CO2 stripping of volatile organic compounds from high gravity beer fermentation

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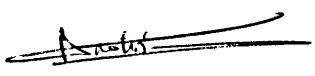

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To Chris

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Summary

An interest has arisen in fermentation techniques using high gravity medium for their potential economical viability in the alcoholic beverage industry. However, high gravity fermentation suffers from ethanol yeast inhibition, which results in incomplete attenuation of the medium. Current methods for ethanol removal suffer from expensive investment and loss of flavour and aroma qualities in the final beverage. Gas stripping has been investigated by other workers, as an ethanol removal technique, primarily in non-beverage fermentations using ex-situ extractant gas. Its potential with alcoholic fermentation arises from the naturally evolved CO_2, a source of free extractant. Huxtable [1993] obtained interesting results when using gas stripping as a technique for producing low-ethanol cider in tower fermenters.

In the present study, the gas stripping technique was further investigated with small-scale fermenters and exogenous CO_2, with a view to improve the fermentation of high gravity beer wort. An examination of the gas stripping method for the in-situ removal of ethanol from high gravity beer fermentations was carried out. The effects of CO_2 stripping on the change of the flavour balance of the finished beer were investigated. The technique was also applied to synthetic mixtures containing ethanol and other beer volatile compounds in order to determine the effectiveness of the technique and the relative volatility of the different flavour compounds.

Continuous removal of ethanol through stripping maintained medium ethanol levels below 8% v/v, the yeast ethanol tolerance. When used in conjunction with high gravity fermentation (OG 1080 and 1100), CO_2 stripping reduced by approximately 50% medium ethanol level. The increased rate of fermentation and sugar consumption with gas stripping was attributed to a combination of the removal of yeast inhibition and the enhanced mixing. The number of yeast cells in suspension in the stripped medium increased along side yeast viability, cell size, biomass and budding. However, despite the higher yeast metabolism, net production of ethanol and other flavour-active compounds was reduced. This apparent reduction was attributed in part to partial condensation of the volatile compounds extracted during gas stripping. The changes in isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde were intimately linked to ethanol changes. Their rate of extraction by CO_2 stripping was proportional to their relative volatility. The collected beer condensate provided a natural source of flavour compounds. Its concentration by pervaporation delivered a means of adding back selected compounds in view to equilibrate or enhance the flavour profile of the depleted-stripped beer.

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Legend

Symbols

c	Concentration ($kg.m^{-3}$)
γ	Activity coefficient
p	Partial pressure (atm)
f°	Fugacity (atm)
P°	Pure vapour pressure (atm)
P	Total pressure (atm)
N	Mass transfer rate ($kg.m^{-3}.s^{-1}$)
k	Mass transfer coefficient ($m.s^{-1}$)
a	Gas/liquid interfacial area ($m^2.m^{-3}$)
α	Relative volatility
x	Liquid mole fraction
y	Vapour mole fraction
β	Enrichement factor
w	Mass fraction
H	Henry's Law constant
K	Kvalue, Equilibrium constant

Subscripts/Superscripts

L	Liquid
G	Gas
i, j	Components i and j
v	Volatile
perm	Permeate
∞	Infinite dilution

Abbreviations

°P	Degree Plato
SG	Specific Gravity
GC	Gas Chromatography
MS	Mass Spectrometry
FID	Flame Ionisation Detector
FPD	Flame Photometric Detector
SPME	Solid Phase Micro-Extraction
ATD	Automated Thermal Desorption
EMP	Embden-Meyerhof-Parnas (pathway)
TCA	Tricarboxylic Acid (cycle)
NADH	Nicotinamide Adenine Dinucleotide (reduced form)
VOC	Volatile Organic Compound
PDMS	Polydimethyl siloxane
VLE	Vapour-Liquid-Equilibrium
ABE	Acetone-Butanol-Ethanol
IBE	Isopropanol-Butanol-Ethanol

CHAPTER 1 - INTRODUCTION

1.1 BACKGROUND

Recently there has been an increasing interest in high gravity brewing involving high substrate concentrations, in an attempt to improve ethanol yields. High gravity beers can be diluted with water in order to obtain a standard beer. The use of high gravity fermentation can therefore increase production capacity without having to expand existing brewing plant. For this economic reason, most major brewing companies world-wide are trying to accommodate high gravity brewing in their production processes. However, under the conditions of high gravity fermentations, the yeast are exposed to a variety of environmental stresses resulting from high osmotic pressure and high ethanol concentration. Yeast ethanol inhibition results in poorly attenuated beer, where residual sugars remain at relatively high concentrations. The brewing industry is, therefore, continually seeking for simple and economic techniques which would ferment high gravity medium without premature cessation of yeast activity. A means of decreasing the exposure of the yeasts to toxic ethanol concentration is to extract ethanol as it is formed during the fermentation. Among the various techniques for product removal, gas stripping, which involves diffusion of ethanol from a liquid phase into a gas phase, has been found to be a simple and efficient technique in non-beverage fermentation processes (Park and Geng [1992]). As CO_2 is naturally produced by alcoholic fermentations, it provides a clean and cost effective source of extractant. While it is evolved during the fermentation, it can be utilised and recirculated through the fermentation medium. This technique was used by Huxtable [1993] to produce low-alcohol ciders containing 2.9% v/v ethanol. In addition, preliminary experiments using high gravity cider medium suggested that stripping could result in a higher net ethanol production. The interesting results obtained by Huxtable [1993] led us to investigate the technique further in view of fermenting high and very high gravity beer medium.

1.2 AIMS AND SCOPE

The main objective of this thesis is to investigate the feasibility of CO_2 stripping for the fermentation of high gravity beer. To this effect, an experimental study of gas stripping with small-scale 10 L fermentations using exogenous CO_2 was completed. The effect of CO_2 stripping on the rate of fermentation, the uptake of sugars, the

production of ethanol and other flavour compounds, the production of biomass and the physiology and morphology of the brewing yeast cells was evaluated. Additionally, the influence of agitation and aeration on the stripped fermentations was investigated. To determine the effectiveness of the technique and the relative volatilities of the different beer flavour compounds, the gas stripping process was also applied to synthetic mixtures of chemicals. The UNIFAC model of vapour-liquid equilibrium was used to predict the equilibrium gas phase chemical concentration, in order to determine the relative volatility of beer flavour compounds. Pervaporation was also investigated as a means of concentrating the beer condensate, recovered during stripping.

The subject matter of this thesis is presented from a biochemical and analytical point of view. It is hoped that the experimental results will contribute to more simple and economical techniques of fermenting high gravity worts for the brewing and other alcohol beverage industries.

1.3 THESIS STRUCTURE

Chapter two contains a review of the literature concerning the volatile organic compounds produced during beer fermentation and their production though yeast metabolism, the cause for ethanol inhibition during fermentation, a review of the fermentation processes which utilise gas stripping as a product removal technique, and the potential of gas stripping in alcoholic beverages.

Chapter three details the apparatus, analytical techniques and procedures selected for experimental work.

Chapter four investigates the use of the UNIFAC method to assess the volatility of the major beer flavour compounds, and the efficiency of the condensation unit used during stripping of synthetic mixtures and beer fermentations.

Chapter five includes both results and a discussion related to the use of CO_2 stripping with high gravity beer fermentations. The effect of gas stripping on the level of ethanol produced during the fermentation, the uptake of sugars, the fermentation rate

(measured as a change in specific gravity), medium pH, yeast growth, cell viability, biomass and budding was investigated.

Chapter six presents an overall conclusion of the present study and suggests future work.

CHAPTER 2 - LITERATURE REVIEW

2.1 INTRODUCTION

Brewing is one of the most widely appreciated biotechnology processes known to man. Beer is produced by the fermentative action of brewing yeast on wort, which is an extract of malted barley flavoured with hops. During primary brewery fermentation several yeast mediated biochemical events occur including the production of biomass as a result of cell proliferation and the production of ethanol and flavour active metabolites. The modern malting and brewing industry now applies a whole spectrum of new technical, biochemical, microbiological and genetic inventions. One of the new technologies that industry has focused on is the use of high gravity brewing. High gravity brewing (original gravity between 1060-1078) and very high gravity brewing (original gravity above 1078) has been progressively introduced into breweries around the world for the past twenty years. The major advantage of this process is that by concentrating the mash, increasing production demands can be met without expanding the existing brewing, fermenting, and storage facilities. High gravity brew can then be diluted down to the required ethanol level. Changing the fermentation process i.e. using high gravity wort, can result in an unbalance of the different flavour compounds and the diluted beer can require an adjustment of some flavour chemicals. Therefore, the brewer needs a clear understanding of the mechanisms for the production of the flavour-active compounds, first to assess how a change in the fermentation parameters will affect the final flavour and, second, to be able to apply a suitable change in the fermentation process. The major drawback of high gravity brewing itself is incomplete fermentation due mainly to ethanol inhibition. Product inhibition has been recognised to be a major problem in fermentation processes using a high initial sugar concentration. Considerable efforts have been expended into the search for techniques of ethanol removal from non-alcoholic fermentation medium (Park and Geng [1992]). Within those techniques, gas stripping has been recognised for having great potential in the removal of ethanol during alcoholic fermentation.

2.2 VOLATILE ORGANIC COMPOUNDS IN BEER

The flavour and aroma of beer are very complex, due to the large number of compounds arising from various sources. Some of the beer constituents derive from

the wort, surviving the brewing process unchanged. Barley and malt provide burnt, smoky, nutty and malty tastes while the hops provide bitterness and hoppy flavours. Other beer compounds are the result of chemical and biochemical transformations of the raw materials during malting, mashing, boiling, fermentation and conditioning. However, the compounds responsible for beer flavour are mostly derived from yeast metabolism, which yields ethanol and carbon dioxide plus a large number of other compounds such as higher alcohols, esters, acids and sulphur compounds. The quantities of these compounds are very small compared to ethanol, but because of their low flavour threshold, they markedly affect the organoleptic quality of the final product. All the flavour compounds are produced in response to environmental changes affecting yeast growth and survival. In other words, a change in the natural environment of the yeast by changing the fermentation process will result in different levels of flavour-active compounds and ultimately affect the perceived quality of the final product. It is therefore important to understand the biochemistry taking place inside a yeast cell during a brewery fermentation, to be able to fully appreciate the effect of varying the fermentation process.

2.2.1 Beer active-flavour compounds

The main differences in flavour between alcoholic beverages come from differences in the quantitative analysis of chemicals rather than in the qualitative profile (Suomalainen and Lehtonen [1978]). Ethanol is the major volatile organic compound of alcoholic fermentations in terms of mass fraction. Ethanol produces a warming effect, contributes to perceived sweetness, reduces apparent acidity, gives body and has an overall smoothing effect on other taste characteristics (Williams [1972]). Ethanol is also considered as a flavour enhancer. Sensory examination of a cider extract (Williams and Rosser [1981]) indicated that between 0.5% and 0.75% of ethanol enhanced the fruity character of the aroma. It was suggested that part of this enhancement was a physicochemical effect of ethanol on the vapour pressure of other volatiles. The following sections will give an overview on the most important flavour-active compounds in beer after ethanol. The main compounds are presented in Table 2-1 along with their typical concentrations in commercial beers and their flavour thresholds. The flavour (taste) threshold is defined as being the lowest

Table 2-1: Main volatile organic compounds in beer (Table compiled using Hough et al.[1982], except where indicated).

Chemical group	Volatile compound	Concentration in beer (ppm)	Flavour threshold (ppm)	Organoleptic properties
Alcohols	Ethanol	3-10% v/v	14000	Alcoholic, solvent-like
	Isoamyl alcohol	28-169	65	Alcoholic
	Active amyl alcohol	8-41	70	Alcoholic
	Isobutanol	6-98	200	Alcoholic
	Propanol	5-60	800	Alcoholic
	2-phenyl ethanol	19-55	125	Rose-like
	Furfuryl alcohol	1.2	3000	
Aldehydes	Acetaldehyde	0-33.8	10	Grassy, apple-like[2]
	Furfural	25	150	
Esters	Ethyl acetate	8-69	33	Light fruity, solvent like
	Isoamyl acetate	0.4-4.9	1.6	Banana, pear drops
	Isobutyl acetate	0.03-0.4[1]	1.6	
	2-phenylethyl acetate	1.62	3.8	
	Ethyl caproate (ethyl hexanoate)	0.95	0.23	Apple-like with aniseed notes
	Ethyl caprylate (ethyl octanoate)	1.5	0.9	
	Ethyl caprate (ethyl decanoate)	0.19	1.5	
Ketones	Diacetyl	0.02-0.58	0.15	Sweet, butterscotch[2], buttermilk
	Pentane-2,3-dione	0.01-0.26	0.9	Sweet, butterscotch[2]
Acids	Acetic acid	57-145	175	Vinegar, pungent
	Propionic acid	1.3-5[1]	150	
	Butyric acid	0.62	2.2	Rancid butter
	Valeric acid (pentanoic acid)	0.03	8	
	Caproic acid (hexanoic acid)	2.5	8	
	Caprylic acid (octanoic acid)	6.1	13/15	Soapy, fatty, goaty, tallowy
	Capric acid (decanoic acid)	0.70	10	
	Dodecanoic acid	0.11	6.1	
Sulphur compounds	Hydrogen sulphide	0.45 ppb	30	Rotten egg
	Sulphur dioxide	500 ppb	20000	Striking-match, choking, sulphurous
	Dimethyl sulphide	0.25 ppb	33	

[1]Maarse and Visscher [1989], [2]Hammond [1986]

concentration at which the flavour is perceptible. A similar definition is applicable for the aroma (odour) threshold.

2.2.1.1 Higher alcohols

After ethanol, the largest group of volatile constituents are the higher alcohols. Higher alcohols have a longer carbon chain than ethanol. They have higher boiling points than ethanol and are potently aromatic, exerting a considerable influence on beer flavour and aroma. They are sometimes named fusel alcohols because they are found in the fusel oil, the higher alcohol fraction remaining after the distillation of ethanol from a fermented liquid (Hough *et al.* [1982]). Most of the higher alcohols have an alcoholic or solvent like aroma and, like ethanol, produce a warming effect. The major higher alcohols found in beer are 3-methyl butanol (isoamyl alcohol), 2-methyl butanol (active amyl alcohol), 2-methyl propanol (isobutanol), propanol and phenyl ethyl alcohol. The flavour threshold of these alcohols ranges from about 65 ppm for isoamyl alcohol to 800 ppm for n-propanol. Most of the higher alcohols are present in beer but at levels below their taste threshold, although their levels vary considerably with beer type.

2.2.1.2 Esters

The most important flavour active compounds in beer are the esters, which impart fruity flavours and have relatively low taste thresholds, ranging from 0.23 ppm for ethyl caproate to 33 ppm for ethyl acetate (Hough et al. [1982]). These levels are often reached in beer. Among the many esters (over 3700) present in beer, ethyl acetate is the major one followed by the ethyl esters. Acetates of the higher alcohols (the so-called "banana esters") are also very important. Esters undoubtedly contribute to the overall flavour of beer, but abnormally high levels are regarded as off-flavours, which can destroy the flavour balance of the final product.

2.2.1.3 Aldehydes

Only low levels of aldehydes are found in beer, as they are normally reduced to their corresponding alcohols. However, the major aldehyde is acetaldehyde, which is a metabolic branching point. Acetaldehyde has a grassy, apple-like taste and can be present in beer at close to its flavour threshold (Hammond [1986]). The presence of aldehydes is sometimes due to the oxidation of higher alcohols by melanoidins during the storage of bottled beer. High levels of these aldehydes produce stale off-flavours.

The cardboard flavour of stale beer is thought to be due to 2-trans-nonenal and 2-methylfurfural.

2.2.1.4 Vicinal diketones

Ketones, like aldehydes, are carbonyl compounds but they are not major fermentation products. Those in beer are probably derived from hop oil or hop resin degradation products (Hough *et al.* [1982]). However, vicinal diketones such as diacetyl and pentane-2,3-dione are potent flavouring agents and result from yeast metabolism. They have similar sweet and butterscotch flavours but diacetyl is the most detectable of the two having a taste threshold of 0.15 ppm, which is one tenth that of 2,3-pentanedione. Diacetyl is characteristic of some ales but is undesirable in lagers and stouts. Quantities in excess of 0.5 ppm of diacetyl are regarded as off-flavours in lager beers.

2.2.1.5 Acids

The principal volatile acid of beer is acetic acid, a product of the oxidation of acetaldehyde. Although less important than esters, fatty acids can have important flavour effects on beer. Amongst many volatile acids, fatty acids with an even number of carbon atoms predominate, due to their mode of biosynthesis. Hexanoic (C6), octanoic (C8) and decanoic (C10) acid accumulate during the fermentation and account for 90% of the total beer fatty acids (Hammond [1986]). At the levels exceeding their taste threshold (8-15 ppm), they will impart a soapy fatty flavour to beer. Fatty acids are commonly associated with the yeasty flavours produced after long storage at high temperature. The non-volatile acids, derived from both the malt and yeast metabolism, contribute to the overall beer flavour. In particular, pyruvic, succinic, acetic and lactic acid, produced by yeast metabolism, can affect the acidity and the perceived bitterness due to a lowering of the pH (Hammond [1986]).

2.2.1.6 Sulphur compounds

Compounds such as hydrogen sulphide and sulphur dioxide have very low taste thresholds (30 ppb) and while desirable in trace amounts, they can cause unpleasant odours (Hammond [1986]). Dimethyl sulphide (DMS) is also very important for its

sulphury note. It is part of the characteristic flavour spectrum of lagers but not of ales. Pitching worts contain considerable amounts of DMS, much of which is purged from the fermenter by carbon dioxide evolution. In addition, dimethyl sulphoxide (DMSO) is present.

2.2.1.7 Conclusion

Ethanol, higher alcohols, esters, aldehydes, acids and sulphur compounds constitute the volatile fraction of the flavour-active compounds of beer. A change in the fermentation parameters will change the flavour balance of the final product, by enhancing or decreasing the production of some of these compounds. Ester production, for example, is influenced by yeast strain, pitching rate, temperature, pressure, level of suspended solids, wort aeration and composition. High gravity wort has been shown to lead to excessive production of esters. Production of esters during laboratory-scale fermentations of ale yeasts or lager yeasts was much greater in high gravity worts (19°P) than in normal gravity worts (9.5°P) (Calderbank and Hammond [1994]). Calderbank and Hammond [1994] also showed that the production of esters was regulated by the levels of the corresponding higher alcohols.

2.2.2 Identification and quantification techniques

Qualitative and quantitative analysis of the beer flavour compounds is very important in the brewing industry, as they serve as quality control tools. Gas chromatography (GC) methods for the analysis of alcoholic beverages flavours have been used for many years. Various GC columns have been developed specially for the purpose. Packed columns were first used, but now capillary columns offer improved resolution, permitting more compounds in the same sample to be separated and thus detected. Detectors such as the Flame Ionisation Detector (FID) have been commonly used for the routine quantification of many beer flavour compounds. Specific detectors such as the sulphur-selective Flame Photometric Detector (FPD) have been used to measure volatile sulphur compounds (Leppänen *et al.* [1979]). Mass spectrometry coupled to a gas chromatograph has also become a common tool for identification and confirmation of trace flavour compounds (Sharpe and Chappell [1990]). The main drawback of GC techniques is the necessity of the preparation of a

clean extract, where the compounds of interest must be concentrated and free from interfering substances. An important criteria of the extract is that it must be representative of the original flavour of the beverage. Progress in chromatographic technology now enables the use of a variety of sample preparation techniques. Although each technique serves some specific analytical purpose, they all suffer inherent drawbacks. The main techniques, which have been used to analyse the flavour compounds of alcoholic beverages such as beer, wine and cider, are summarised in Table 2-2.

A method employing direct injection with GC for the determination of ethanol in beer has been tested by the Analysis Committee of the Institute of Brewing (Buckee and Mundy [1993]). Precision, repeatability and reproducibility were acceptable and the method was approved. By using direct injection on a packed column, Clarkson *et al.* [1995] showed an improvement on the repeatability/reproducibility compared to the IOB Recommended Distillation method. However, direct injection of beer samples is only suitable for the measurement of ethanol and the main beer volatile compounds such as isoamyl alcohol, isobutanol and propanol. The actual detectors are not sensitive enough to detect trace compounds, which applies to most of the beer volatile compounds. Concentration methods using distillation, solvent extraction, solid phase extraction and headspace techniques have been developed to increase sensitivity and enable the identification and quantification of trace compounds.

Distillation at atmospheric pressure involves the application of heat, which may create alcoholysis and/or saponification of the esters, together with other artefact formations. Vacuum steam distillation with subsequent solvent extraction of the distillate has been more successful. The solvent is usually ether, pentane or ether-pentane mixture. The major drawback of this technique is the possible incomplete separation by the solvents and the requirement for correction factors due to the differences in extraction coefficients of the various compounds.

Table 2-2: Current sampling techniques used for the determination of flavour compounds in alcoholic beverages.

Technique	Reference	Advantages	Disadvantages
Direct injection	Buckee and Mundy [1993] Clarkson *et al.* [1995]	Rapid and simple, useful for the major compounds	Interferences of non-volatile substances; limit in sensitivity; decreased column life; not useful for trace components.
Distillation		Sample fractionation	Incomplete recovery; thermal decomposition; time-consuming
Solvent extraction - Ethyl acetate - Hexanol - Methylene chloride - Freon 113	Iverson [1994] Alvarez *et al.* [1994] Stenroos *et al.* [1985] Ferreira *et al.* [1993]	Selective, permits sample concentration	Solvent contamination and artefact formation; time consuming; emulsion problems which necessitate centrifugation
Carbon disulphide (CS_2) extraction	Stenroos *et al.* [1976]	Little response to CS_2, enables measurements of peaks normally hidden by other solvents, does not discriminate against low-volatility compounds	Time consuming CS_2 associated with carcinogenic risk
Solid phase extraction -XAD-2 resin / diethyl ether -Kieselguhr / dichloromethane	Hawthorne *et al.* [1987] Irwin and Thompson [1987] Mangas *et al.* [1996]		Artefacts extracted from the solid phase by the solvent
Static headspace	Baker [1989] Buckee [1992] Basette [1984] Alvarez *et al.* [1994]	Quick and accurate, Analysis of trace volatile compounds, simple and representative of the aromas of beer	Large sample size requirement resulting in poor chromatography and column life; discrimination of low-volatility compounds
Dynamic headspace - Chromosorb 105 - Chromosorb 101 - Porapak Q - Tenax GC - Tenax TA	Williams and Strauss [1977] Leppänen *et al.* [1979] Lindsay *et al.* [1972], Williams *et al.* [1978] Noble *et al.* [1979], Chen [1983], Chen [1985] Kaipainen [1992]	Enable pre-concentration of trace volatile compounds.	Interference of water; requires more complex instrumentation and a non-reactive, thermally stable, absorbent trap; discrimination of low-volatility compounds

Continuous liquid-liquid extraction with solvents such as ether, ether-pentane, and isopentane, range over considerable periods, usually from one to several days. Ethyl acetate can be used to extract the fermentation by-products of beer (Iverson [1994]). The method enabled determination of the isoamyl alcohol, ethyl hexanoate, ethyl octanoate, isobutyric acid, phenyl ethyl acetate, phenyl ethyl alcohol but not of isoamyl acetate and ethyl decanoate because of interferences. The ethanol concentration was found to affect the determination of glycerol. Solvent extraction is usually unsatisfactory because it can influence the composition of the aroma extract owing to solvent selectivity and the possibility of artefact development.

Carbon disulfide (CS_2) solvent extraction is well known and widely used (Alvarez *et al.* [1994], Stenroos *et al.* [1976], Stenroos *et al.* [1985]). Different approaches of this technique generally achieve some concentration, as the analytes are collected in a smaller volume than occupied by the original beer sample. Concentration of an extract increases the sensitivity of the technique further, as it enables the detection of compounds present at low levels in the original beer, so that low volatility components are not discriminated against. The disadvantage of the method is that it is time-consuming because of the concentration step and the analytical run time. Moreover, the carcinogenic risk linked with CS_2 makes its use undesirable in modern laboratories.

Solid phase extraction techniques have also been developed with different types of resin. The technique has been extensively used in environmental chemistry, for the extraction of organic compounds from water. Hawthorne *et al.* [1987] applied the technique for the extraction of low molecular weight organic compounds from beer. The simple and rapid procedure involved adsorption of the component onto XAD-2 resin followed by desorption with diethyl ether. The applications of the method, which was reproducible and suitable for almost all capillary GC, included the determination of the major esters, higher alcohols, and fatty acids for both quality control and research and development. Irwin and Thompson [1987] used a commercially available tube of Kieselguhr and elution with dichloromethane. The technique was found to be a much faster (1 hour shorter) and more efficient method than liquid-liquid extraction for obtaining a beer flavour extract. Provided an internal standard is added to the beer prior to extraction, accurate quantitative analysis of the

major flavour components could be performed. Solid-phase extraction with a Kieselguhr tube using GC/MS was developed with the aim of monitoring flavour components of cider (Mangas *et al.* [1996]). Good recoveries for alcohols, esters, lactones, phenols and fatty acids were obtained, however the short-chain acids were not extracted.

Headspace sampling is generally the most appealing technique because of its simplicity and the reduced risk of artefact formation (Chen [1983], Baker [1989]). Headspace is generally defined as the gaseous mixture surrounding a sample within a closed system. In beer the gaseous mixture represents the volatile aroma. The two modes of headspace sampling technique are static withdrawal or displacement. Static headspace has been used for the analysis of fusel oil in beer (Buckee [1992]). In principle, the volatile compounds in beer are "salted-out" in a sealed vessel with sodium chloride or potassium sulphate, and the equilibrium headspace vapour at 30°C is automatically sampled and transferred directly onto a GC column. The transfer may be accomplished directly by syringe withdrawal, plunger displacement, or valve switching under reduced pressure. It is recognised that the method is not ideal, but it provides a good starting point for the analysis of beer volatiles by gas chromatography. Basette [1984] reviewed the errors and limitations of the method, particularly the equilibrium of the sample with its vapour. An adequate equilibrium has to be found with the temperature, the addition of salting-out compounds, the presence of lipids, proteins, acids and bases. The main problem with this sampling mode is the large volume of vapour sample that is required, as each volatile component is very dilute. Unless pre-concentrated, e.g. on a suitable porous polymer, the large sample size may cause poor chromatographic resolution, particularly when capillary columns are used. Moreover, large samples contain large amounts of water vapour, which is detrimental to column life.

These problems have been in part overcome by the introduction of dynamic headspace analysis. This sampling process, also called the "purge and trap" method, has been used extensively for environmental studies, such as detection of organic pollutants in water. In this case, the sample is purged with a non-condensable inert gas that sweeps the volatiles in the headspace onto a trapping adsorbent. The porous polymers trap flavour compounds while allowing the removal of water. The trapped

and enriched volatiles are subsequently recovered by thermal desorption before being introduced to the analytical system. Wohleb [1972] and Lindsay *et al.* [1972] were the first to demonstrate the application of this sampling technique to the collection and concentration of beer flavours by using a prototype discontinuous purge and trap process. Automated and efficient purge and trap samplers have now been introduced commercially. The use of such samplers not only eliminates the drudgery of routine and repetitive manipulations, but also increases the reproducibility of the results because the sampling variables can be controlled more precisely. The success of the dynamic sampling technique is largely dependent on the adsorption and desorption capability of the trap. Several porous polymers have been used as adsorbent traps, including Tenax GC, Porapaks, and the Century series of Chromosorb. Williams and Strauss [1977] used the headspace technique with Chromosorb 105 for the analysis of alcoholic spirits and beverages. The choice among these adsorbents is made according to the specific application. Tenax GC is considered a superior general-purpose adsorbent because of its excellent thermal stability, ease of desorption, non-retention of water, and freedom of background peak. However, the polymer has a relatively small surface area and thus, small breakthrough volume, which is defined as the amount of entrainment gas required to force a compound through the trap. Compounds with lower volatility and smaller molecular size may break through an adsorbent-trap with a smaller amount of purge gas (Chen [1983]). Thermal desorption followed by GC and GC-MS has been successfully used for the analysis of volatile sulphur compounds in beers, wines and distilled beverages (Leppänen [1979]). The aroma compounds of low and non alcohol beer have been qualitatively analysed using thermal desorption and GC-MS by Kaipainen [1992].

The conventional sampling/concentration techniques such as distillation, solvent extraction, solid phase extraction and CS_2 extraction suffer from inherent drawbacks. Advances in headspace techniques enabled their use on a more routine basis. As stated previously, dynamic headspace is the most preferred technique. In combination with GC/MS, it provides an excellent tool for the identification of trace flavour compounds in alcoholic beverages such as beer. Using this technique, a more complete spectrum of beer flavour compounds may be obtained, or a specific flavour defect may be detected, for example, identifying process fault or microbiological contamination.

Solid-phase micro-extraction (SPME), an alternative method to static and dynamic headspace techniques, has been recently explored for the analysis of beer flavour compounds (Jeleń *et al.* [1998]). SPME is a relatively new, simple and inexpensive technique for the isolation of headspace flavour compounds. SPME headspace samplings requires neither solvent extraction and purification steps nor a complicated purge and trap apparatus. The SPME unit consists of a holder and a fused silica fibre, which is coated with a layer of stationary phase such as non-polar poly(dimethylsiloxane) or polar polyacrylate. When a SPME fibre is inserted in the headspace of an airtightly sealed sample bottle, an equilibrium partition process occurs between the sample and the SPME coating. The equilibrium depends mainly on the heating time, temperature, sample volume and sample concentration. The fibre can then be directly inserted in the injector port of a gas chromatograph.

In the present study, a dynamic headspace technique using a Tenax TA adsorbent was developed for the qualitative analysis of beer flavour compounds. Preliminary results for the comparison of the flavour profiles between stripped beer and control beer are presented. Alternatively, direct injection of the beer sample onto a packed column was used to quantitatively analyse both ethanol and the main beer volatile compounds such as isoamyl alcohol, isobutanol, propanol, acetaldehyde and ethyl acetate.

2.2.3 Biochemistry of alcoholic fermentations

The complexity of the composition of beer stems from the various reactions occurring during yeast metabolism. For survival, yeasts cells need a number of basic nutrients. A source of carbon is provided by fermentable sugars present in wort. Most brewing yeasts can metabolise glucose, fructose, sucrose, maltose, and maltotriose (Hough *et al.* [1982]). Pentoses and dextrins survive untouched into the finished beer. The required source of nitrogen is provided by the amino acids and peptides from the malt. The lipids (unsaturated fatty acids and sterols) are necessary for the synthesis of cell membranes and hence are essential for growth. Brewing yeasts can synthesise the lipids providing that an adequate supply of oxygen is present. Small quantities of growth factors such as biotin and panthotenate are vital for some enzyme activities (Jones *et al.* [1981]). The requirements are yeast strain specific and can be met by the levels present in wort. Inorganic ions such as iron, magnesium, sodium and potassium are necessary for yeast growth (Jones *et al.* [1981]) but excessive levels of some (such as copper and iron) can be toxic.

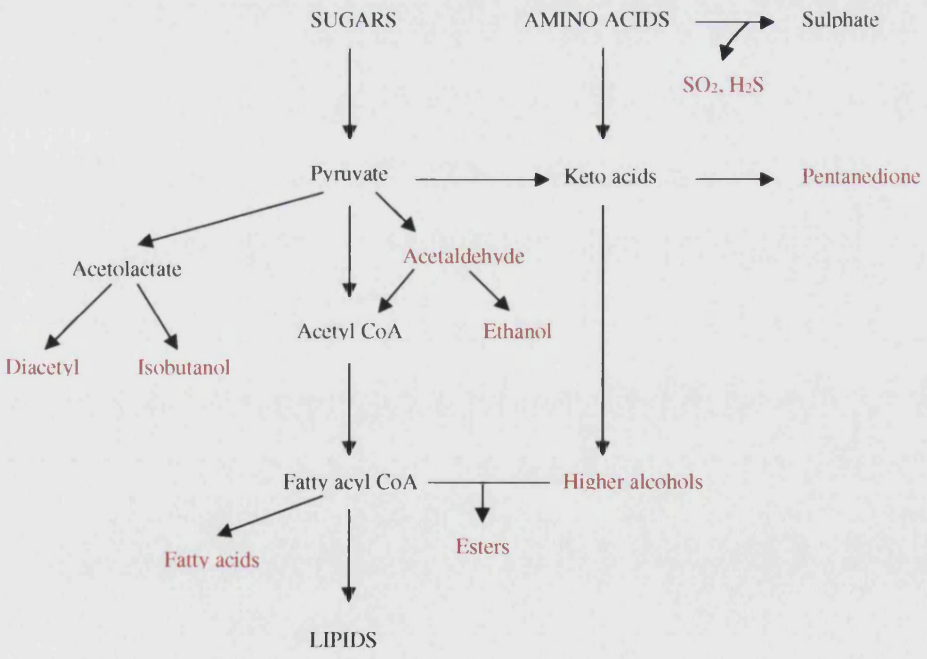

Figure 2–1: The inter-relationship between yeast metabolism and the production of flavour-active compounds (reproduced from Hammond [1986]).

During yeast metabolism, flavour-active compounds are synthesised along side ethanol via different pathways, and play a major role in the flavour of beer. The inter-relationships between yeast metabolism and the production of flavour-active compounds are summarised in Figure 2–1.

2.2.3.1 Production of ethanol

The main biochemical process of the alcoholic fermentation is the break down of glucose into a mixture of ethanol and carbon dioxide, by strains of *Saccharomyces.* The stoichiometry of the catabolic reaction was first described in 1815 by Gay-Lussac and reads as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Glucose enters the yeast cell by way of one or more transport proteins located in the plasma membrane. The main pathway (common to aerobic and anaerobic fermentations) of this biochemical process is glycolysis, also known as the Embden-Meyerhof-Parnas (EMP) route, where one molecule of glucose is converted into two molecules of pyruvate (Figure 2–2). Fructose is phosphorylated by hexokinase to give fructose 6-phosphate which enters the EMP pathway directly. Sucrose is hydrolysed extracellularly to a mixture of glucose and fructose by the enzyme invertase (β-fructosidase) which is a mannan-protein located in the walls of *Saccharomyces.* Starchy raw materials (such as malt) are degraded prior to fermentation to maltose, glucose, fructose and sucrose together with smaller amounts of oligosaccharides including maltotriose and maltotetraose. Maltose and maltotriose are hydrolysed intracellularly to glucose by the yeast enzyme maltase.

Under aerobic conditions, pyruvate would enter the Tricarboxylic Acid cycle (TCA) or Krebs' cycle followed by the electron transport chain. Glucose would be completely oxidised to carbon dioxide and water. However, under the anaerobic conditions of a brewing fermentation, pyruvate produced through the EMP pathway is decarboxylated by the enzyme pyruvate decarboxylase, with the formation of acetaldehyde and carbon dioxide. The acetaldehyde formed acts (in the absence of the respiratory chain) as an electron acceptor and oxidises NADH (produced during

glycolysis) with the formation of ethanol. Pyruvate is not completely decarboxylated into ethanol and carbon dioxide, but a small amount acts as a source of carbon, for the biosynthesis of cell constituents.

At the start of the fermentation when the glucose concentration is in excess of 4 $g.L^{-1}$, glucose is fermented rather than respired even in the presence of dissolved oxygen, due to catabolite repression. The anaerobic pathway leading to the formation of ethanol and carbon dioxide occurs until the sugar concentration has been reduced to a very low level. The catabolite repression of glucose is also known as the Crabtree effect. Once the glucose level has declined to 0.05 $g.L^{-1}$, if oxygen is admitted in the fermentation medium, aerobic respiration, giving complete oxidation of the carbon source begins. After a short lag phase to synthesise the relevant enzymes, the ethanol produced during anaerobic metabolism can be respired aerobically.

In normal circumstances, brewing yeast strains (*Saccharomyces cerevisiae* and *Saccharomyces uvarum*) are capable of utilising sucrose, glucose, fructose, maltose and maltotriose in that order. The major limiting factor in the fermentation of wort is the repressing influence of glucose upon maltose and maltotriose uptake. The yeast cell, when presented with two or more sugars will usually first choose to metabolise the sugar it utilises with greater ease. It is only when 60% of the wort glucose is taken up by the yeast that the uptake of maltose commences (Stewart *et al.* [1988] and Crumplen *et al.* [1989]). In typical industrial brewing, glucose, fructose, and sucrose are consumed within 24-28 hours, compared to maltose, which is consumed between 70 and 72 hours, and maltotriose after 72 hours (Hough *et al.* [1982]). The effect of catabolic repression is more pronounced in high and very high gravity wort. Phaweni *et al* [1992] found that there was a critical concentration of glucose under which the yeast was inhibited, and that the rate of glucose uptake was greatly influenced by the physiological condition of the yeast.

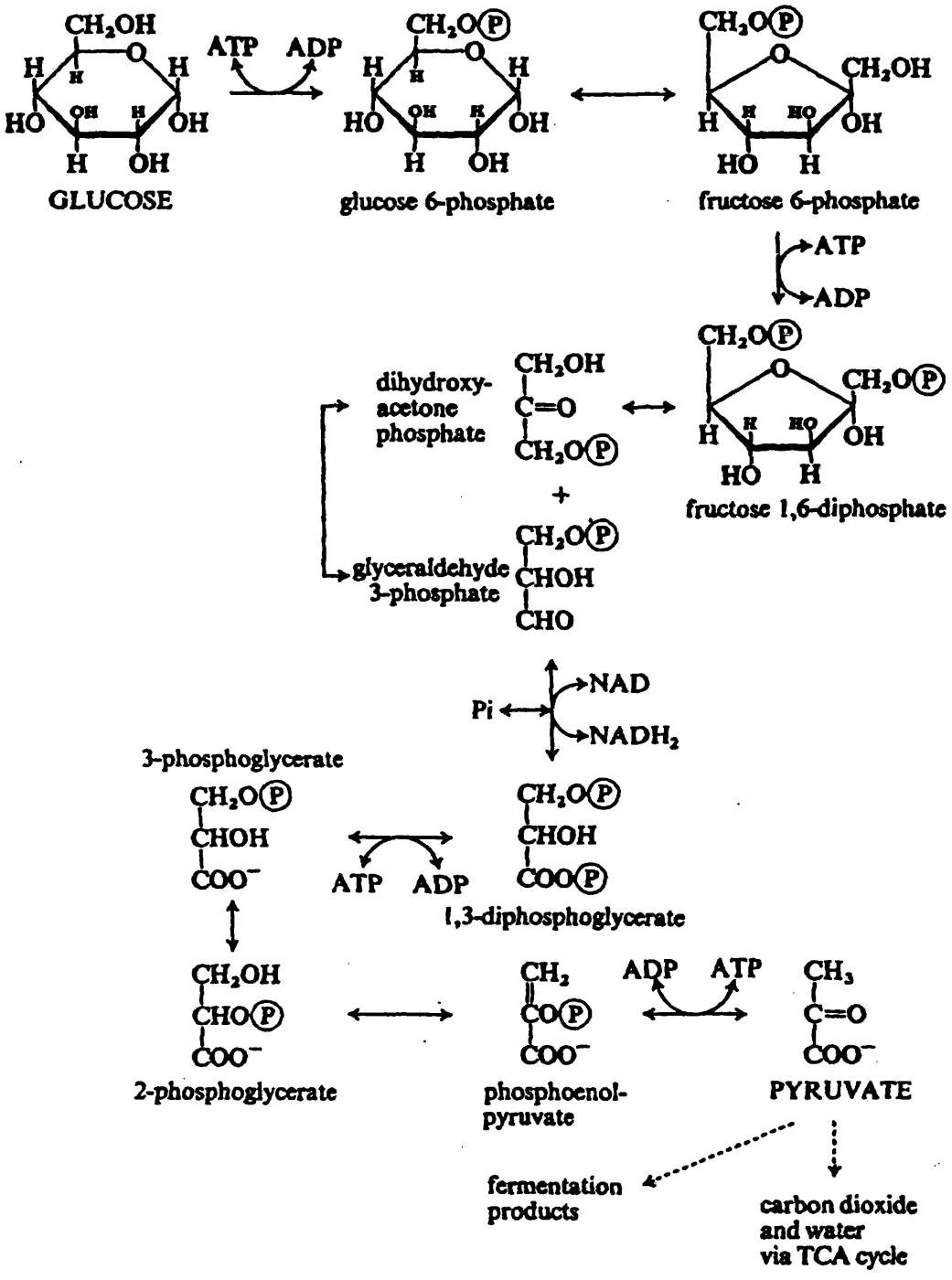

Figure 2–2: Embden-Meyerhof-Parnas pathway for the production of ethanol (reproduced from Hough et al. [1982]).

2.2.3.2 Production of beer flavour compounds

The fusel alcohols are by-products of wort amino-acids breakdown or of amino-acid biosynthesis (Hough *et al.* [1982]). In both cases, α-keto acids are produced, either by transamination of amino acids (referred to as the Erhlich route, or the catabolic pathway) or by synthetic routes from glucose (referred to anabolic pathway). The α-keto acids are then converted into aldehydes through the action of keto acid decarboxylase and in turn to alcohols though the action of alcohol dehydrogenase. The relative contribution of the Ehrlich pathway and the biosynthetic pathway is not yet very clear. Using a label tracer technique using [^{14}C] and [^{3}H], Chen [1978] found that the relative contributions of both pathways vary with each fusel alcohol, that glucose utilisation was relatively small and that the contribution of the biosynthetic pathway decreased with the number of carbons in the fusel alcohol molecule. The interrelationship between both pathways is described in Figure 2–3.

Most of the aldehydes are derived from the α-keto acids, before being reduced to the higher alcohols. Therefore, only low levels of aldehydes will be found in beer, except for acetaldehyde, which is the intermediate between pyruvate and ethanol. Acetaldehyde production is at its maximum during primary fermentation. Because conversion of acetaldehyde to ethanol requires zinc ions, a shortage of the ions can lead to excess acetaldehyde production.

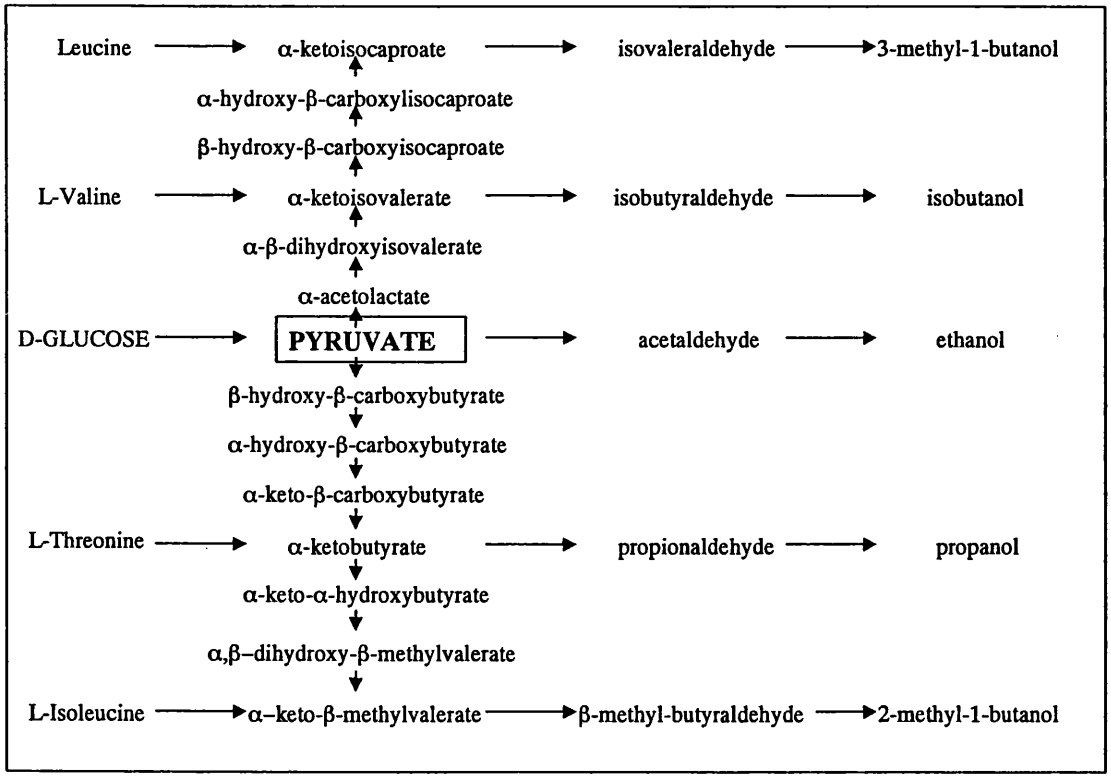

Figure 2–3: Biochemical pathways to the formation of fusel alcohols and their interrelationship (reproduced from Chen E.C.-H [1978]).

The esters are produced intracellularly by the condensation of acyl Coenzyme A (acyl CoA) compounds with alcohols. As the acyl CoA compounds are also intermediates of fatty acid synthesis, the production of esters is therefore closely linked with lipid metabolism. The alcohols involved in the alcoholysis are ethanol and the higher alcohols. Early in the fermentation, lipid synthesis is required for cell growth, and therefore the specific rate of ester production is very low. Later, when yeast growth is restricted by oxygen supply (e.g. in high gravity brewing), cells are unable to synthesise unsaturated fatty acids and sterols, and hence cease to grow. Under these situations, providing that adequate amino nitrogen is present in the wort, ester synthesis increases dramatically and then tails off towards the end of fermentation. When high gravity wort (above OG 1060) is fermented, disproportionate amounts of esters are formed, so that dilution to normal gravities will produce very estery beers. At high gravity, yeast growth will become limited in the presence of high concentrations of assimilable nitrogen and of the products of fermentation, such as higher alcohols. At normal nitrogen and gravity levels, various physical factors can have an effect on ester synthesis. This increases with temperature and decreases with

agitation and pressure. The effect of pressure is probably due to carbon dioxide poisoning (Jones and Greenfield [1982]).

The acids are formed by the fatty acid biosynthesis pathway from acetyl coenzyme-A. Their formation is regulated in much the same way as esters, the levels decreasing with increasing oxygenation, the addition of unsaturated fatty acids or the dilution of assimilable nitrogen by sugar addition.

The vicinal diketones (diacetyl and 2,3-pentanedione) are formed non-enzymatically in the medium by decarboxylation of acetohydroxy acids secreted by yeasts. The acetohydroxy acids involved, α-acetolactate and α-acetohydroxybutyrate (intermediates of amino-acids biosynthesis) are formed by the condensation of hydroxyethyl thiamine pyrophosphate with the oxo-acids, pyruvate and α-oxobutyrate respectively (Figure 2–4). High temperature and reduced pH tend to favour diacetyl formation, the removal of which depends on yeast activity and on the temperature. An active yeast is required for the conversion of diacetyl into much-less flavour active compounds such as acetoin and 2,3-butanediol (Hammond [1986]).

Sulphur compound production is also related to the amino-acid metabolism of the yeast. Hydrogen sulphide is generated during yeast metabolism, and its maximum rate of production coincides with the maximum rate of yeast growth. In normal brewery worts, the hydrogen sulphide arises from organic sulphur compounds (cysteine and methionine), either from the metabolism of those present in the wort or from the breakdown of yeast proteins. Thus, cysteine from either source encourages H_2S production through the action of the enzyme cysteine desulphydrase. In the absence of organic sulphur compounds, hydrogen sulphide arises from sulphate ions. Dimethyl sulphide (DMS) is derived either from malt via a heat-labile precursor, S-methyl methionine or produced during fermentation from the enzymic reduction by yeast of dimethyl sulphoxide (DMSO). DMSO is a product of the oxidation during malt kilning and wort boiling of DMS. H_2S and DMS may also arise as a result of the metabolic processes of contaminant micro-organisms. Many of the thiols and mercaptans found in beer may be derived from hops and other materials rather than from yeast metabolism.

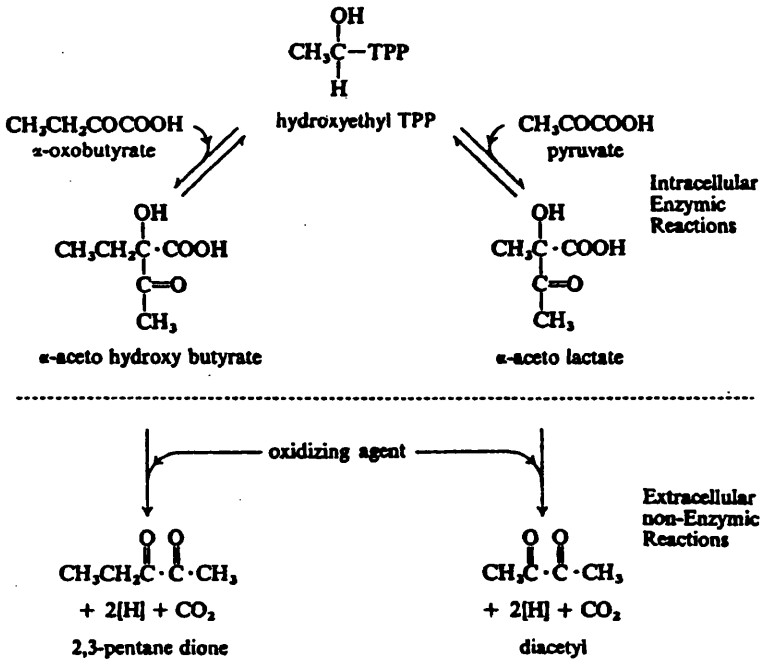

Figure 2–4: The formation of α-acetohydroxy acids and their non-enzymic oxidative decarboxylation to diacetyl and 2,3-pentanedione (reproduced from Hough et al. [1982]).

2.3 INHIBITION OF YEAST FERMENTATION BY ETHANOL

The fermentation of sugars in the brewing industry is generally carried out with yeast producing 4 to 9% (w/v) ethanol. The amount of ethanol produced is limited by the inhibitory effect of ethanol on the particular yeast cells. The presence of ethanol at the inhibitory level in the medium results in premature cessation of yeast activity. In high gravity fermentations containing a high concentration of sugars, ethanol inhibition will result in poorly attenuated wort, where sugars remain at relatively high concentrations. Ethanol tolerance of *Saccharomyces* yeast is not simply a result of the intrinsic ability of different strains to tolerate and produce differing levels of ethanol. Tolerance is dramatically influenced by the nutritional condition of the wort, as well as by the environmental factors. The mechanisms of ethanol inhibition and the different parameters influencing ethanol tolerance have been reviewed by Casey and Ingledew [1985], Casey and Ingledew [1986], D'Amore and Stewart [1987] and Stewart *et al.* [1988b]. The following sections summarise their findings.

The assessment of ethanol tolerance in various yeasts is very difficult, as there is no accepted technique of measurement or definition of ethanol tolerance. Ethanol has three major effects on yeast cells. It inhibits cell growth, cell viability and fermentation rate. Methods for defining ethanol tolerance have been developed using these three parameters. The most widely employed method for determining ethanol tolerance involves the suppression of cell growth in the presence of exogenous ethanol. The major drawback of this method is that the medium used can influence the degree of ethanol tolerance. Another method measures the ratio of the rates of fermentation in the absence of ethanol compared with those containing high levels of ethanol. However, ethanol tolerance is not expressed in terms of ethanol concentration but rather in rate of fermentation. The third method defines ethanol tolerance to maximum amount of ethanol resulting from sugar fermentation. It has been proposed that the best indicator of ethanol tolerance was the inhibition of fermentative ability.

There have been many mechanisms proposed to explain the inhibitory effects of ethanol. These include denaturation and inhibition of glycolytic enzymes, inhibition of glucose, maltose, ammonium and amino acid transport, depression of the optimum

and maximum temperature for growth and an increase in the minimum temperature for growth, damage to the cell membrane resulting in altered membrane organisation and permeability, accelerated passive re-entry of protons in a manner resembling the action of an uncoupler, and the enhancement of chemical death and "petite" mutation in yeast. The yeast cell plasma membrane has been found the primary site of ethanol toxicity. The plasma membrane is the site controlling the transport of nutrients into the cell and the excretion of waste products (i.e. ethanol) into the surrounding medium. Plasma membrane phospholipids and sterols have been shown to play an important role in the ethanol tolerance mechanism. The most common fatty-acyl residues of phospholipids are palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1 n-9) and vaccenic acid (C18:1 n-7). The fatty-acyl residues of phospholipids appear to maintain the fluidity of the plasma membrane. Ergosterol, which is the major sterol in yeast is regarded as fulfilling a structural role. Because ethanol and plasma membrane lipids are both amphiphatic molecules, they might interact during a fermentation causing physical and chemical changes in the membranes. It is not yet clear if ethanol increases or decreases the fluidity of the plasma membrane, as published results are contradictory. However, the specific composition of the membrane in lipids was shown to influence ethanol tolerance. It was found that the increase in fatty-acyl chain length and in the proportion of unsaturated fatty acids and sterol found in the membrane resulted in increased ethanol tolerance. Supplementation of growth media with various unsaturated fatty acids, vitamins and proteins were shown to enhance ethanol tolerance. Casey and Ingledew [1985] supplemented a high gravity wort (27°P) with a mixture of yeast extract (1%), ergosterol (40ppm) and Tween 80 (oleic acid) (0.4%). With the supplementation, fermentation time decreased from 2 weeks to 4 days. Reduction in the fermentation time was the result of a dramatic increase in the duration and level of cell mass synthesis arising from nutrient supplementation, which overcame wort nitrogen and unsaturated fatty acids deficiencies. It follows that in high gravity brewing, in order to have rapid fermentation, both the length and level of new cell mass synthesis must be increased above the amounts found in normal gravity brewing.

In brewery fermentations, the primary factors limiting the production of high levels of ethanol by brewers yeasts are a combination of nutritional deficiencies in unsaturated lipids and assimilable nitrogen. O'Connor-Cox and Ingledew [1991]

found that the use of high pitching rates overcame the deficiency in assimilable nitrogen in a high gravity (16°P) lager fermentation by increasing the fermentation rate. Oxygen is required by the brewers yeasts for the synthesis of sterols and unsaturated fatty acids. Under the anaerobic conditions of a brewery fermentation, brewing yeasts are not able to synthesize the sterols and unsaturated fatty acids. In high gravity worts, oxygen solubility is diminished even further due to the increase in sugar concentration. Since growth (and rapid fermentation rates) ceases once a limiting value of unsaturated lipids is reached in the yeasts, the low oxygen solubility in high gravity worts increases the probability of the growth-related attenuation problem. This results in stuck or sluggish fermentations. To obtain both normal yeast growth and a satisfactory fermentation pattern, the required lipids must be added to the wort, or oxygen must be made available for their synthesis. In a study on the effect of the timing of oxygenation on very high gravity fermentations with *Saccharomyces uvarum*, O'Connor-Cox and Ingledew [1990] found that oxygen was the most stimulatory when it was added between 10 or 14 hours post-pitching. A high gravity (25°P) wort was successfully fermented by D'Amore [1992] by increasing the yeast pitching rate, the initial oxygen level and the temperature. The beer produced by the 25°P wort fermentation was diluted to 5% (v/v) ethanol and compared with a standard 16°P wort fermentation. The finished beers had similar organoleptic characteristics.

There is now rising interest in high gravity brewing involving high substrate concentrations, in an attempt to improve ethanol yields. However, high substrate concentrations have been shown to inhibit yeast growth and fermentation as a result of high osmotic pressure and low water activity. It has been observed that with an increase in the osmotic pressure of the medium, yeast viability and fermentative ability decreased due to accumulation of high levels of intracellular ethanol (D'Amore and Stewart [1987]). To counteract the rise in external osmotic pressure yeast produces glycerol. High osmotic pressure inhibits the diffusion of produced ethanol to the external medium, which creates a toxic build-up of intracellular ethanol by plasmolysis. To avoid the rise in osmotic pressure and therefore maintain cell viability, methods such as substrate feeding by sequential addition during the fermentation have been employed. To increase the osmotolerance of yeast, Crumplen

et al. [1990] employed the spheroplast fusion technique. The technique was successful in fusing dextrin-fermenting yeasts with osmotolerant yeasts, and resulted in higher fermentation rates and extent, osmotolerance and thermotolerance compared with the parent strains and with several brewing yeasts.

High fermentation temperature and high osmotic pressure have a similar effect in reducing ethanol tolerance. Yeast can respond to the physical effects of high temperatures (increased membrane fluidity) by changing their fatty acid composition. Unlike the effect of ethanol, with increasing temperature the proportion of saturated fatty acids (primarily palmitic and myristic acids) esterified into membrane lipids increased at the expense of unsaturated acyl chains (linoleic acid). Associated with that, was a considerable decrease in the quantity of membrane phopholipids. This decrease in fatty acid unsaturation with increasing fermentation temperature serves to maintain optimal membrane fluidity for cellular activities. As is the case with osmotic pressure, increasing fermentation temperature has been shown to cause accumulation of intracellular ethanol. Ethanol has also been shown to enhance the lethal effects of high temperatures (thermal death) and decrease the maximal temperature permitting growth. The effect of heat shock and ethanol stress on the viability of a lager brewing strain during the fermentation of high gravity wort was studied by Odumeru *et al.* [1992]. Cells were found less tolerant to heat shock during the fermentation of high gravity wort (25°P) than with normal gravity medium (16°P). Relieving the stress effects of ethanol by washing the cells improved their tolerance to heat shock. Thus, cells in the presence of high concentrations of ethanol develop increased sensitivity to heat shock.

Increase in fermentation temperature and osmotic pressure has both been correlated with increased intracellular concentration of ethanol and decreased ethanol tolerance of yeast cells. It was initially reported that exogeneous ethanol is less toxic than endogeneous ethanol produced by the yeasts. This finding was thought to be due to the rate of ethanol production within the cells exceeding the rate at which it could be excreted. It has been observed that the ethanol concentration inside the cell can be greater than outside at certain stages and under special environmental conditions. The general assumption has been that because of its small molecular size and solubility in membrane lipids, ethanol transports rapidly across biological membranes in response

to a concentration gradient. Stewart *et al.* [1988] found that ethanol accumulates in the very early stages of fermentation (> 6 hours) but very quickly equilibrates so that intracellular and extracellular concentrations are similar for most of the fermentation. However, it has also been reported that the intracellular concentrations of ethanol in fermenting suspensions of yeast are less or equal to those in the extracellular environment. The discrepancy in results is most likely due to problems with the accuracy of the techniques employed to measure intracellular ethanol concentrations. Until an accurate and universal method is defined, the role of intracellular ethanol accumulation in ethanol toxicity and tolerance will remain uncertain.

Ethanol toxicity is the main factor limiting increased ethanol production in brewing, and more particularly in high gravity brewing. It is expected that, providing all the essential nutrients are made available in the fermentation medium, high gravity wort could be fully fermented by continuous removal of ethanol. Simple and cost effective methods for product removal are needed. The present study has explored the used of gas stripping for the removal of ethanol.

2.4 PRODUCT REMOVAL BY GAS STRIPPING

Gas stripping has been investigated in many non-alcoholic fermentations such as ethanol, ABE (Acetone-Butanol-Ethanol) and IBE (Isopropanol-Butanol-Acetone) fermentations, where the main objective was to remove product inhibition in order to increase the reactor productivity. In alcoholic fermentations, gas stripping is a relatively new technology, and has been primarily investigated as a method of dealcoholisation by Huxtable [1993]. Gas stripping will be compared with traditional separation techniques, and also, with more recent techniques such as pervaporation. A review of the different applications of gas stripping is presented after outlying the basic principles of gas stripping.

2.4.1 Basic principles of gas stripping

Gas stripping, also known as desorption, refers to the transfer of volatile components from a liquid to a gas phase, which is the reverse operation of gas absorption. Perry *et al.* [1984] give the following definition: "Gas absorption is a unit operation in which

soluble components of a gas mixture are dissolved in a liquid. The inverse operation, called stripping or desorption, is employed when it is desired to transfer volatile components from a liquid mixture into a gas."

A volatile organic compound (VOC) is an organic compound, which has volatility of one or greater at room temperature, relative to water. Relative volatility is defined by $\alpha=(y_i/x_i)/(y_j/x_j)$, where x_i, x_j are liquid mole fractions and y_i, y_j vapour mole fractions of components i and j respectively. Relative volatility can also be considered as the selectivity of vapour liquid equilibrium systems, in comparison with the selectivity defined for example in pervaporation by $\beta=(w_i/w_j)^{perm}/(w_i/w_j)^{feed}$ (Baudot and Marin [1997]), where w_i and w_j are mass fractions of components i and j respectively.

Equilibrium represents the limiting condition for any gas-liquid contact. The driving force for mass transfer from a liquid phase to a gas phase can be defined simply as the difference between the actual and equilibrium conditions at any point in the liquid phase. Many models have been proposed to explain and correlate mass transfer from a liquid phase to a gas phase. Although the film theory is in fact an inexact representation of conditions at the gas-liquid interface, it is the most widely accepted and has proved to be an effective correlation tool. It is commonly applied to the design of absorption and stripping equipment, and particularly in environmental studies. Gas stripping of low-concentrations volatiles, such as air stripping of volatile contaminants from drinking water (Kavanaugh and Trusssel [1980]), is usually modelled under steady state conditions by the two-film theory. The same approach can be readily adapted to describe CO_2 stripping of volatiles from fermenting beer.

The two-film theory, illustrated in Figure 2–5, is based on the fact that the gas and liquid phases are in equilibrium at an interface and thin films separate the interface from the main bodies of the two phases. The bulk of the liquid and gas phases are assumed to be well mixed, while little or no fluid motion occurs within the films and the process of molecular diffusion becomes the primary mechanism of mass transfer. The bulk gas and liquid concentrations are clearly not equilibrium values, since otherwise net diffusion of the solute would not occur. It is assumed that there is no resistance to solute transfer across the interface separating the phases, and consequently the concentrations at the interfaces are equilibrium values.

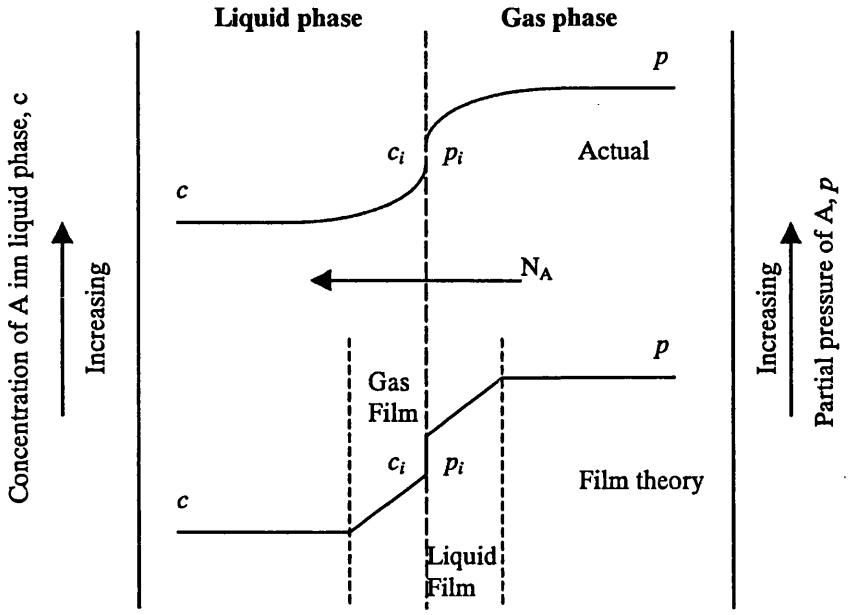

Figure 2–5: The two-film theory concept for an absorption system. c_i and p_i represent equilibrium conditions at the interface (reproduced from Rousseau [1987]).

Two mass transfer coefficients are defined then as k_L, the quantity of material transferred through the liquid film per unit time, per unit area, per unit of driving force in the liquid, and k_G, the quantity transferred through the gas phase per unit time, per unit of gas-phase driving force. A material balance across the interface yields the following simple relationship:

$$N_A = k_L(c - c_i) = k_G(p_i - p)$$

N_A = quantity of component A transferred per unit time, per unit area
p = partial pressure of A in the gas bulk
p_i = partial pressure of A in the gas at the interface
c = concentration of A in the liquid bulk
c_i = concentration of A in the liquid at the interface

For experimental determination of the rate of mass transfer, it is usually impossible to determine the solute concentrations, c_i and p_i at the interface. As a result, it is customary to define c*, a theoretical liquid-phase concentration which is the concentration which would be in equilibrium with the partial pressure, p in the bulk gas, and p*, a theoretical partial pressure which would be in equilibrium with a

solution having the composition c. c* and p*, equilibrium concentration and partial pressure can be determined using UNIQUAC or WILSON non ideal package.

Consequently, the rate of mass transfer can be defined using overall coefficients K_G (overall gas mass transfer coefficient) and K_L (overall gas and liquid mass transfer) by the following relationship:

$$N_A = K_L(c - c^*) = K_G(p^* - p)$$

The valued for K_L and K_G incorporates the diffusion to resistance to mass transfer in both phases and is related to the local gas and liquid mass transfer coefficients k_G and k_L, respectively, and its Henry's Law constants, H by the following relationships:

$$\frac{1}{K_G} = \frac{1}{k_G} + \frac{H}{k_L}$$

$$\frac{1}{K_L} = \frac{1}{Hk_G} + \frac{1}{k_L}$$

For very soluble solute where the Henry's constant is low, the term H/k_L is much smaller than $1/k_G$ so that $1/K_G \sim 1/k_G$. In such a case, the gas film represents the controlling resistance, and mass transfer data can be correlated best in terms of K_G. When a constituent has a relatively large Henry's Law constant, such as the volatile components in the beer wort, the K_L and K_G will be dominated by resistance to transfer through the liquid film (k_L), such that $K_L \sim k_L$ and $K_G \sim k_L/H$. Consequently the mass transfer will be best correlated in terms of K_L.

The rate of mass transfer will usually include a term for the interfacial area per unit volume of contactor. For the CO_2 stripping of volatile compounds from a fermenting wort, the rate of volatile mass transfer across the wort-CO_2 interface will therefore be related to the volatile's liquid concentration as follows:

$$Nv = K_L a(c_v - c_v^*)$$

Where, N_v is the volatile mass transfer rate ($kg.m^{-3}.s^{-1}$); c_v^*, the volatile equilibrium concentration in the wort ($kg.m^{-3}$); c_v, the actual volatile concentration in the beer ($kg.m^{-3}$); K_L, the overall liquid mass transfer coefficient ($m.s^{-1}$), and a, the CO_2/wort interfacial area per unit volume of wort ($m^2.m^{-3}$).

2.4.2 Gas stripping with non-alcoholic fermentations

Gas stripping, either in-situ or in a fermenter side loop, has been exploited to remove volatile components from active fermentations in a variety of non-beverage processes, mainly in the ethanol, ABE and IBE fermentations. It has been also investigated for the production of glycerol (Kalle *et al* [1985]) and fatty acid ethyl esters (Morin *et al.* [1994]). Product removal techniques such as gas stripping have been extensively used in fermentation processes to reduce product inhibition, in order to increase substrate consumption, therefore improving productivity.

2.4.2.1 In the ABE fermentation

Ennis *et al.* [1987] evaluated gas stripping, an adsorbent resin, and a molecular sieve (silicate) for solvent removal. Gas stripping was the most successful technique because it removed only volatile solvents, and not essential nutrients. In-situ N_2 stripping for the removal of toxic butanol from batch fermentation using *Clostridium acetobutylicum* P262 was investigated by Ennis *et al.* [1986]. Solvent recovery from the gaseous phase was achieved by condensation in a cold trap. The selectivity (relative volatility) of the butanol/water separation at equilibrium was found equal to 19. The solvents (butanol, acetone and ethanol) productivity and lactose consumption improved with product removal via gas stripping compared to control fermentation without product removal. It was concluded that in-situ gas stripping for solvent recovery by condensation could be used to selectively remove toxic butanol from fermentation broths and to achieve a significant increase in fermentation performance.

Batch ABE fermentation was further studied in a novel fermenter with in-situ solvent removal with gas stripping by Duffy [1988] . The effects of gas flow rate and sparging gas composition (N_2, CO_2) on product spectrum and the efficiency of separation was investigated. In-situ removal of solvents while maintaining reasonable yields and good substrate utilisation was claimed to be possible. However, the removal of solvents

achieved in the gas phase was very low compared to their production. Two explanations are offered; low solvent concentration in the liquid phase and low stripping gas flow rate (maximum gas flow rate used was 0.4 $L.L^{-1}.min^{-1}$ compared to 2.74 $L.L^{-1}.min^{-1}$ used by Ennis *et al.* [1986]). Duffy [1988] findings were followed up by Mollah [1990], who investigated the same system in a continuous fermentation, which enabled a constant medium composition. The main results of this study were summarised by Mollah *et al.* [1993]. In-situ gas stripping was capable of keeping the butanol concentration under the toxic level (5 $g.L^{-1}$), but was not suitable for complete removal of solvents from the broth. Solvent productivity was found to be proportional to sparging gas flow rate up to 0.67 $L.L^{-1}.min^{-1}$ of gas, and then decreased as the gas flow rate increased further due to shear on biomass.

The use of an external stripper rather than in-situ stripping has also been investigated. A 2L fermenter coupled with an external stripper using N_2 as a stripping gas has been used by Groot *et al.* [1989]. The fermentation medium was stripped of solvents by the external stripper and recycled to the reactor. In batch fermentations, the substrate consumption was increased threefold using in-situ gas stripping compared to a control fermentation without in-situ recovery. For continuous fermentations, in-situ recovery led to an increase in biomass concentration, resulting in a threefold increase in productivity. The selectivity of the butanol/water separation was equal to 4, which is low compared to 19, the selectivity at equilibrium (Ennis *et al.* [1986]). This was due to incomplete recovery of butanol by the condensation unit, the design of which was not optimised. Qureshi and Maddox [1991] used a fluidised bed reactor coupled with an external stripper. Cells of *Clostridium acetobutylicum* were immobilised in the reactor by adsorption onto Bonechar. The major results were improved lactose utilisation (due to the relief of product inhibition), improved solvent yields and high reactor productivity. At a dilution rate of 1.37 h^{-1}, a reactor productivity of 5.1 $kg.m^{-3}.h^{-1}$ was achieved.

As fermentation removes as much as 40-50% of the consumed sugar on a carbon basis, other researchers have investigated the reuse of fermentation gas rather than using an external source of gas. Park *et al.* [1991] investigated the use of fermentation gas with an immobilised cell trickle reactor. The immobilised cell reactor separator (ICRS) consists of two glass columns. In the enricher, the trickling liquid is in cocurrent contact with fermentation gas, and in the stripper the liquid is in countercurrent contact with gas

upflow. After circulation in the enricher and in the stripper, the fermentation gas was passed to the product recovery unit (absorber), and the solvents were recovered via absorption in water. Cells were immobilised on polyester sponge strips, which were fixed on iron mesh screens. Butanol removal was as efficient as acetone removal in spite of butanol's high boiling point (117°C at atmospheric pressure), due to butanol's high activity coefficient at dilute concentrations as found in fermentations. Stripping of organic acids was far less efficient than of solvents. Up to 87.4, 37.3 and 18.3 % of butanol, butyric acid and acetic acid respectively were recovered by the water absorber. With this removal of toxic products, glucose conversion improved by 33.6 % and 54.7 % at feed glucose concentrations of 60 $g.L^{-1}$ and 80 $g.L^{-1}$ respectively. Numerical calculations predicted that glucose concentrations higher than 80 $g.L^{-1}$ could be converted, but this could not be shown experimentally because of increased cell degeneration.

Maddox *et al.* [1995] have also investigated the recycle of fermentation gas (CO_2 and H_2) in a 2L glass bioreactor. The gases were pumped at 1.5-3.3 $L.min^{-1}$ through the fermentation medium, and the vapours were condensed. Using the simultaneous product removal process, lactose concentrations up to 200 $g.L^{-1}$ could be fully fermented, and the productivity reached 0.32 $g.L^{-1}.hr^{-1}$ compared to 0.07 $g.L^{-1}.hr^{-1}$ without product removal.

2.4.2.2 In the ethanol fermentation

The validity of using evolved carbon dioxide gas to strip ethanol has been initially demonstrated using a two-stage immobilised cell reactor by Dale *et al.* [1985]. Gaseous carbon dioxide has been used to continuously strip ethanol from fermentation media in the small-scale laboratory production of non-beverage ethanol (Walsh *et al.* [1983]). Initially CO_2 from a cylinder was pumped through the fermenter and then through a condenser at 0°C to remove ethanol before re-circulation. As CO_2 was produced by the fermentation, the supply from the cylinder was progressively reduced and finally stopped, the volume of CO_2 eventually being self-sufficient. The CO_2 carrying ethanol was also passed through columns packed with activated carbon to adsorb the ethanol. It was estimated that for a 95% stripping efficiency, one gram mole of ethanol could be removed by 34 gram moles of CO_2.

Gas stripping can also be used in the production of ethanol by fermentation in continuous fermenters (Taylor *et al.* [1995], [1996]). The contents of 2L or 14L fermenters were recycled through a stripping column, as a means of reducing product inhibition and lowering the cost of fuel ethanol production. Complete conversion of 200 $g.l^{-1}$ and 600 $g.l^{-1}$ glucose feed respectively, was achieved in a small pilot plant. Ethanol was recovered from the carbon dioxide stripping gas in a condenser. Productivity of ethanol as high as 15.8 $g.L^{-1}.hr^{-1}$ and condensate production of up to 10 $L.day^{-1}$ of almost 50% v/v ethanol were maintained for up to 60 days.

2.4.2.3 Conclusion

In processes where distillation is adopted for concentration after fermentation, an initial concentration of solvents by gas stripping could potentially result in energy savings when further concentrated by distillation. Qureshi and Maddox [1991] found that the condensed solvents from the stripping gas gave an ABE solution of 53.7 $kg.m^{-3}$, which was seven times higher than that of the reactor effluent.

In a review on separation techniques for extractive fermentation, Park and Geng [1992] compared gas stripping with traditional separation techniques such as vacuum fermentation, liquid-liquid extraction, aqueous two-phase system, adsorption and with more recent techniques such as pervaporation and perstraction. Gas stripping was recognised as having great advantages over the conventional techniques. Two of the most established techniques, liquid-liquid extraction and adsorption, suffer major drawbacks. Liquid-liquid extraction has the potential for energy saving in the recovery of fermentation products compared to distillation. However, liquid extractants, found to be non toxic to microorganisms, possess poor distribution coefficients, and therefore large quantities of extractant are needed. The required amount of solid adsorbents is also very large. Furthermore, adsorption is disadvantageous because it removes intermediate products (organic acids) along with products (alcohols), and can also remove nutrients and sugar. Gas stripping shares an advantage of clean product separation with pervaporation and perstraction. Non-volatile products such as glycerol and organic acids as well as nutrients and cells are not removed by either of these techniques. A disadvantage of membrane based techniques is the low product flux which can be overcome by reducing membrane

thickness and by increasing contact time. Liquid membranes and hollow fibres have been developed to overcome this problem. Since perstraction requires alcohol recovery from extractants, pervaporation would seem to be the most promising technique of the two. Membrane based techniques (pervaporation and perstraction) have an advantage over traditional phase equilibrium processes, in such that they can dramatically increase product selectivity. Gas stripping is not so selective to alcohols as pervaporation using solvent selective membranes, because the selectivity of gas stripping is determined by gas-liquid equilibrium. However, unlike pervaporation, mass transfer for gas stripping is not limited by the diffusion rate through the membrane. Mass transfer can be increased by improving gas-liquid contact mode (countercurrent contact of trickling liquid with the gas stream). For a large scale operation, gas stripping is probably the most attractive process because of its relative simplicity and low capital cost. The stripping gas does not have to be purchased because fermentation converts as much as 40-50% of the consumed sugar on a carbon basis.

2.4.3 Use of gas stripping with alcoholic beverages

Gas stripping was utilised for the production of reduced ethanol cider by Huxtable [1993]. The use of gas stripping in alcoholic beverage fermentation is quite a novel approach compared to its application in the ethanol and ABE fermentations. As opposed to the current methods of dealcoholisation, gas stripping does not require a post-fermentation treatment and does not require great capital investment. Current methods of dealcoholisation for producing low-alcohol beverages have been reviewed by Muller [1990], Stein [1993] and Kulandai [1994]. The latest review was composed by Scott and Huxtable [1995] and is summarised in Table 2-3.

Table 2-3: Current techniques for the production of depleted-alcohol beverages reviewed by Scott et al. [1995].

	Technique/process	Product	Reference	Major outcomes
Post-fermentation removal	Vacuum distillation	Ethanol depleted ciders and wines	Schobinger [1982]	High capital cost and considerable maintenance. Boiled-off flavour
	Membrane techniques - Reverse osmosis - Dialysis	Reduced ethanol wines (6-7% v/v)	Bui *et al.* [1986]	Highly demanding in energy. Expensive membrane, Membrane fouling, other flavour compounds removed with ethanol. Expensive Loss of flavour and aroma
	Freezing	Low ethanol wine	Veila [1984]	
	Supercritical CO_2	Low ethanol wine (1%v/v)	Carbonell [1991]	Clean technique but expensive.
Manipulated fermentation	Dilution of high gravity fermentations	Low ethanol beer (2%)	Kavanagh *et al.* [1991]	Loss of volatiles (higher alcohols, esters, free fatty acids)
	The Barrel Patent	Low ethanol beer	Muller [1990]	Claimed to exhibit the flavour of a high gravity fermentation.
	Temperature (Cold contact process)	Ethanol free beer < 0.05%	Schur [1983]	
	Arrested batch fermentation	Low ethanol beverages	Bulin [1984], Villetaz [1986], Dzionziak [1989]	
	Arrested continuous fermentation	Low ethanol beer 0.1-0.3% Low ethanol beer 0.55%	Aivasidis *et al.* [1991] Van de Winkel *et al.* (1991)	Claimed to have the same levels of fusel oil and esters as a normal beer Claimed to reduce the undesirable worty taste and aroma.

Methods for the production of depleted and ethanol-free beverages have been assigned to two broad categories; post-fermentation removal and manipulated fermentations. In the post-fermentation removal techniques, fermentation goes to completion and ethanol is extracted from the finished beer at the end of the process. Manipulated fermentations are fermentations where ethanol production is suppressed during the course of fermentation. Manipulated fermentations have been the most economical way of producing low-alcohol beverages but have led to undesirable changes in taste and flavour profile of the product. Manipulated fermentations are often characterised by a worty taste and aroma. This has led to the development of much more expensive post fermentation processes such as vacuum distillation, membrane separation and supercritical CO_2 extraction. Recent advances in dialysis systems have made this method considerably more attractive than reverse osmosis. Dialysis plants have been successfully installed in a small number of breweries around the world. Although it operates successfully, its potential has not been exhausted yet and more research is being carried out (Leskošek and Mitrović [1994], Leskošek *et al.* [1995]). In addition to the requirement for capital investment and the extra processing time and energy, the post removal techniques suffer from the lack of selectivity towards the removal of ethanol, and result in the loss of flavour-active compounds such as the higher alcohols and esters. Consequently, it is often an integrated part of the process to return a fraction of the extract to the dealcoholized beverage, in order to avoid the production of an overly bland and unpalatable beverage. An additional process is often therefore required to fractionate the extract from the ethanol through distillation and therefore results in a higher operating cost. Therefore manipulated fermentations have received much attention in recent years, and have focused on the understanding of the resulting changes in yeast metabolism. As an example, the production of alcohol-free beer (Van Iersel *et al* [1999]) by immobilisation of *Saccharomyces* in a packed-bed reactor, was optimised by introduction of aerobic periods to stimulate yeast growth. Despite the cost disadvantages of post-removal fermentations, research also continues into the further development of these techniques. Supercritical CO_2 extraction has been further investigated by Medina and Martinez [1997] for the dealcoholisation of cider using a pilot-plant equipment. Gomez-Plaza *et al.* [1999] studied the dealcoholisation of wine by vacuum distillation, and focused more specifically on the recovery of the different

volatile compounds lost through the process. A new method for the dealcoholisation of wine using solid carbon dioxide has been investigated by Antonelli *et al.* [1996]. Wines were stripped of alcohol by spraying them over solid phase CO_2. Ethanol contained in the gas leaving the solid-liquid contact zone was condensed and recovered. Ethanol, along with some higher alcohols, was removed, but the other volatile compounds remained at similar levels as in the untreated wine.

Continuous removal of ethanol by gas stripping during the course of a fermentation can be classified as a manipulated fermentation technique, as ethanol is extracted as it is produced. Gas stripping offers considerable advantages over the current methods of dealcoholisation, as it does not require a post-fermentation treatment or need massive capital investment. A first investigation of the technique for dealcoholisation of cider was carried out by Huxtable [1993]. Gaseous CO_2 is a copious natural by-product of fermentation, and hence represents a large potential reservoir of extractant. Evolved CO_2 was therefore used as the stripping gas and continuously recycled through the fermentation medium. Not only did continuous CO_2 stripping enable the production of a depleted-ethanol beverage (2.9 % v/v) but it maintained ethanol concentration below the yeast inhibitory level such that sugar conversion was improved. The continuous removal of a fraction of the fusel alcohols did not result in a dramatic fall in their concentration in the stripped medium. It was also found that the total quantities (medium and condensate) of ethanol, isobutanol, isoamyl alcohol, and ethyl acetate were modified when using stripping and depended on fermentation temperature and original gravity (Scott and Cooke [1995]).

Following the findings of Huxtable [1993] and Scott and Cooke [1995], it was therefore anticipated that ethanol removal by gas stripping was not just an efficient technique for the dealcoholisation of beverages, but could be used to fully ferment high gravity wort. Consequently, the main focus of the present study was the use of gas stripping as an ethanol removal technique in order to remove ethanol inhibition and improve sugar consumption of high gravity beer wort. If fully fermented, the high gravity medium (where the whole extracted condensate or part of it would have been added back) could be diluted back to a standard level of ethanol.

CHAPTER 3 - MATERIALS AND METHODS

3.1 EXPERIMENTAL APPARATUS

Small-scale fermentations were carried out in 10 litre glass culture vessels (Fisher scientific) (Figure 3-1). Each fermenter was linked with gas tubing to a coil condenser (operated at 0±2°C) to recover volatiles carried over in the gas stream. Four fermenters were set-up, two acting as controls (no gas circulation) and the other two operated with regulated addition of CO_2 through a ring-sparger located at the base of the fermenter. The stainless steel ring-spargers were perforated with approximately 20 holes, of approximately 1mm diameter. CO_2 was supplied from a high-pressure cylinder and passed through a sterile PTFE filter (0.2 μm) before entering the fermenter. CO_2 flow rate was controlled with a valve flowmeter operating between 0 and 5 $L.min^{-1}$. Waste gas was released to the atmosphere. The medium temperature was controlled via immersion of the vessels in a large water bath fixed at 22°C or 16°C. Mixing of the medium was carried out by gentle circulation at 0.26 $L.min^{-1}$ with a peristaltic pump (Watson-Marlow 503U). Aeration of the wort was achieved by injecting air at 1 $L.min^{-1}$ for 75 minutes until 44% saturation was reached. When required, the coil condenser operating at 0°C was extended by an additional condensation step constituting a glass trap, maintained at -40°C using a mixture of dry ice and acetone. A food grade antifoam 1520EU (Dow Corning, Reading, UK) was added to the medium before stripping was initiated.

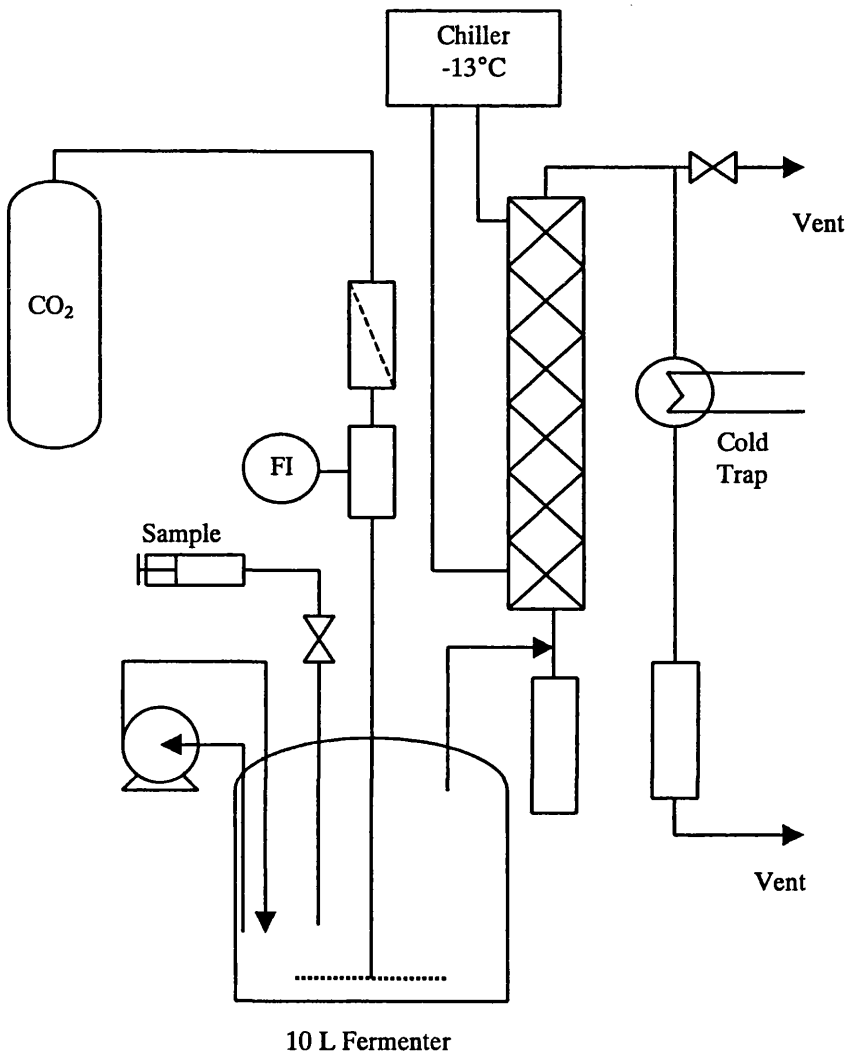

Figure 3–1: Schematic diagram of the laboratory scale fermenter.

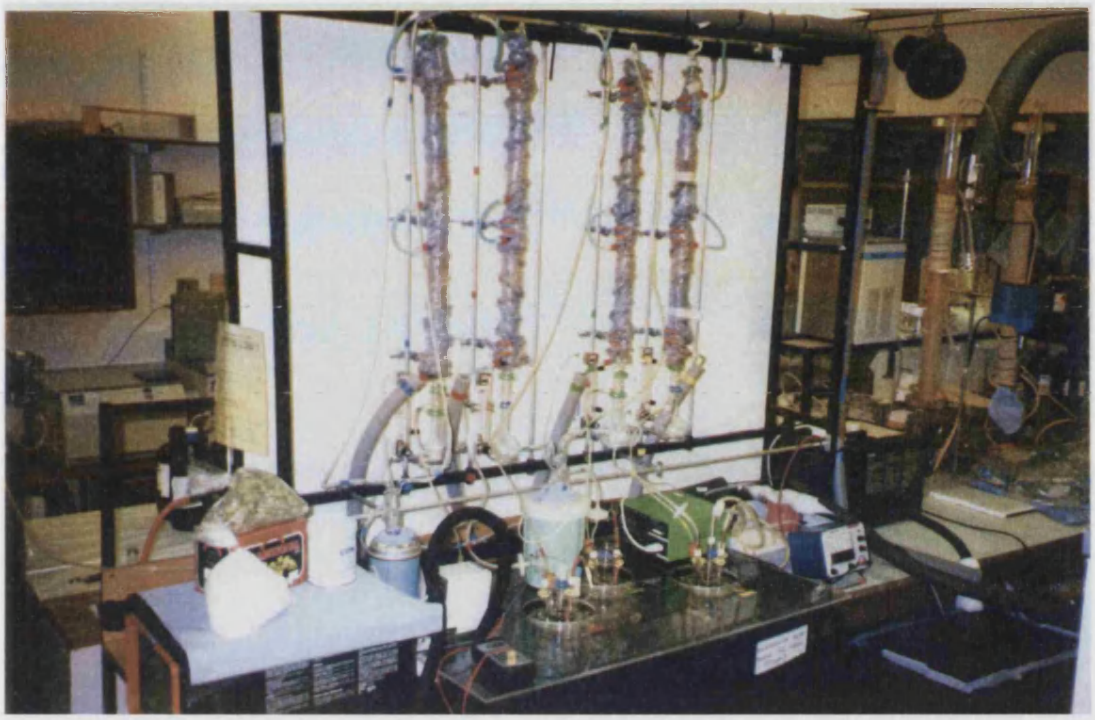

Figure 3–2: Experimental apparatus for the study of CO_2 stripping of high gravity beer fermentations.

3.2 FERMENTATION MEDIUM

3.2.1 Yeast culture

The majority of the fermentations were carried out with an ale strain of *Saccharomyces cerevisiae*, NCYC 1236, which specifications are presented in Appendix A. The yeast strain was supplied on an agar slope by the National Collection of Yeast Culture (Norwich, UK). Occasionally when the NCYC 1236 species was not available, LA-1, a wine strain of *Saccharomyces cerevisiae* from the University of Bath culture was used. The yeast slopes were stored as freeze-dried cultures and subcultured every 3 months.

3.2.2 Starter culture

A fresh starter culture, obtained in two steps, was prepared prior to the start of each fermentation experiment. A volume of 100ml of YPD medium (Difco laboratories, USA) was inoculated with a loop-full of yeast culture and incubated aerobically for 24 hours at 30°C and 150 rpm. A 400mL volume of beer medium, of original gravity 1050, was prepared in a 1 L flask using the recipe described in Table 3-1. The beer

medium was then inoculated with the 100ml inoculum and incubated for another 24 hours. At the end of the incubation time, the 500ml starter-culture contained approximately 10^8 cells.ml^{-1}.

3.2.3 Beer fermentation medium (wort)

3.2.3.1 Ingredients

For lack of a commercial brewing formulation (i.e. provided by a local brewery), a homebrewing kit was chosen for its relative ease of preparation. Beer medium was prepared using a Young's Beer kit (Young's Homebrew Ltd., Bilston, UK), containing spray malt, hops and barley grains. In addition to the beer kit, sugar was supplied as a Brewing Liquid Sugar mixture (Edme Ltd., Manningtree, UK). The recipe supplied with the beer kit was manipulated by increasing the malt concentration for the preparation of high-gravity worts. The original gravity of the medium was varied by altering the amount of sugar added to the medium, keeping constant the concentrations of malt, hops and barley grains, as shown in Table 3-1. Small-scale experiments, using 200 mL of beer wort, were carried out to determine the relationship between specific gravity and sugar concentration. Figure 3–3 show the results obtained when varying the Brewing Liquid sugar from 0 to 120 g.L^{-1}. An extrapolation to the higher specific gravity of 1080 and 1100 was carried out to obtain the required sugar concentration. High gravity of 1080 and 1100 corresponds approximately to 19°P and 23°P respectively. Degree Plato (°P), unit usually employed in industrial brewing, is the % extract in the original wort, and corresponds to the concentration in % w/w of a sucrose solution. Conversion tables from specific gravity measurement to °P can be found in Hough *et al.* [1982].

Table 3-1: Formulation of beer wort for different original gravities.

Ingredient	Concentration (g.L^{-1} of wort)		
	OG 1050	OG 1080	OG 1100
Spray Malt	70	70	70
Hops	5.25	5.25	5.25
Barley grains	11.25	11.25	11.25
Brewing liquid sugar	**95**	**180**	**240**

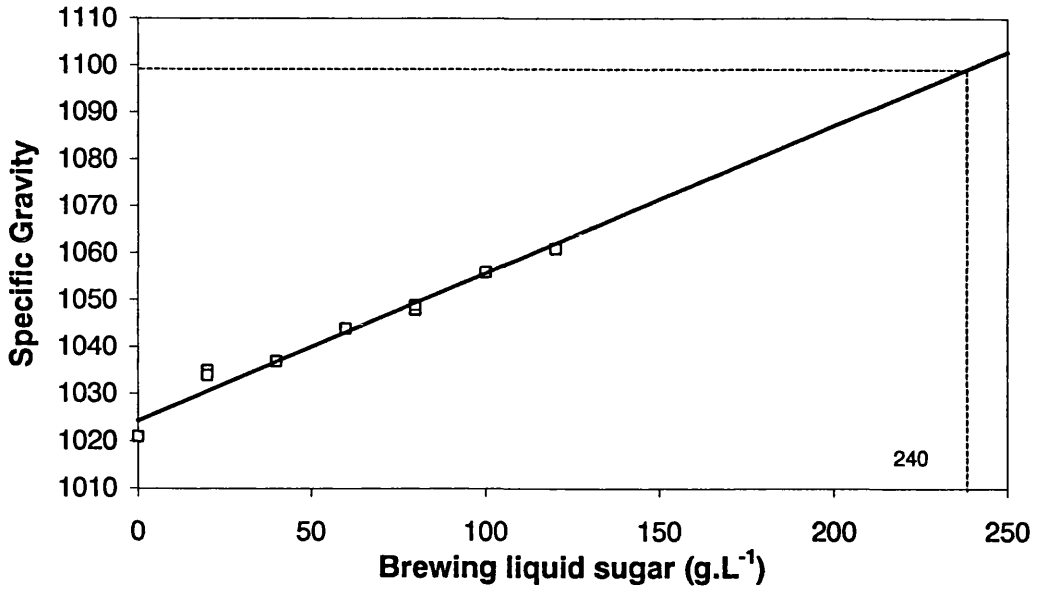

Figure 3–3: Relationship between specific gravity and sugar concentration. The concentration of malt was kept constant at 70 g.L^{-1}.

3.2.3.2 Sterilization

All glassware, tubing and ancillary equipment were autoclaved at standard conditions of 121°C for 20 minutes. Vessels containing large quantities of water (3-7 litres) were autoclaved at standard temperature but for 40 minutes.

3.2.3.3 Preparation of concentrated beer wort

Beer medium was prepared in a 30 litre vessel. Sterile water (17.5% of the total volume) contained in the vessel was brought to its boiling point. Malt was poured carefully into the boiling water and well mixed by hand with a spatula. The malt mixture was simmered for 15 minutes and hand mixed at regular intervals with a spatula. Hops and barley grains were placed in a straining bag, and introduced into the hot mixture, which was then simmered for an extra hour. The straining bag containing hops and grains was then removed and the sugar was added, while mixing continuously. The concentrated wort was then simmered for a further five minutes.

3.2.3.4 Small scale fermentations

An equal volume of concentrated wort was aseptically dispensed into the 10 litre sterile fermenters containing approximately 5 litres of sterile water. More sterile water was added to make-up to the desired volume. The 10 litre fermenters were placed into a water bath at a controlled temperature. When the beer medium reached the desired temperature, it was then inoculated with 100ml of starter culture, as prepared in section 3.2.2 and well stirred. Fermentation was enabled to start with an initial cell concentration of approximately 10^6 cells.mL^{-1}.

3.3 ANALYTICAL METHODS

3.3.1 Fermentation sample collection

A total fermentation medium volume of 60 mL was removed daily from each fermenter with a sterile syringe. The first 10 mL was discarded, and the remaining 50 ml sample was used for analysis. A volume of 1 ml was used for cell counts and viability assessment. The rest of the sample was centrifuged twice at 4000 rpm for 10 minutes using a refrigerated bench-top koolspin centrifuge (Biotech instruments Ltd., Luton, UK). Liquid supernatant was filtered through a 0.45 μm membrane (Nalgene), and stored frozen for specific gravity, pH, sugar, ethanol and other beer volatile compounds analyses. The cell pellet was used for biomass determination. Extra fermentation samples were collected for cell sizing and SEM analyses when required.

Whole condensate samples were collected daily from the stripped fermentations and frozen once weighed.

3.3.2 Specific gravity

The specific gravity was measured using a digital density meter (DMA35, Paar) which was calibrated with distilled water at 20°C before each use.

3.3.3 pH

The pH was measured using a bench-top pH meter (ATI Orion Model CE95, Orion Research Inc., Beverly MA). The pH probe was calibrated before each use with standards of pH 4 and 7.

3.3.4 Cell counts and viability

Changes in cell number and cell viability in the fermentation medium were assessed microscopically using a Neubauer haemocytometer. The fermentation broth was diluted 1:10 in a Ringers buffer, and mixed with Methylene Blue, with a 2:1 dilution. The dead cells take-up the vital stain and appears blue whereas living cells remain non-coloured. The diluted samples were pipetted onto the two counting chambers of a haemocytometer and an average between the duplicate counts of viable and total cell counts was made.

The concentration of cells per mL and percentage viability were calculated as follows:

$$cells.mL^{-1} = average\ total\ cell\ count\ per\ square \times dilution\ factor \times 10^4$$

$$\%viability = \frac{average\ viable\ cell\ counts}{average\ total\ cell\ counts} \times 100$$

3.3.5 Cell sizing

The size distribution of yeast cells in the fermentation broth was determined using a laser Mastersizer (Malvern instruments Ltd). The technique used relies upon the scattering of light by the yeast cells. Large particles scatter at low angles, whereas small particles scatter at high angles. The scattered light is detected and related to a distribution of particle diameter. The Mastersizer calculates cell diameters using the assumption that the cells are spherical and individual. However, budding of the cells produces an irregular shape that may account for a wider size distribution. Only a few drops of the undiluted fermentation samples were needed, and added to a flow cell containing distilled water.

3.3.6 Biomass determination

Cell pellets obtained by centrifugation of the 49 ml samples were used for cell mass determinations. The pellets were washed twice with 40 ml Ringer's solution and then resuspended to 10 ml of the same solution. Duplicate 3 ml samples of the concentrated solutions and of the resuspending Ringer's solution were transferred to pre-weighed aluminium pans. The pans were dried to constant weight at 105°C and the cell dry weight per ml of fermenting liquid was calculated following correction for the weight of the Ringer's solution.

3.3.7 Scanning electron microscopy

Scanning electron microscopy (SEM) can provide detailed pictures of the morphology of yeast. A T330 microscope was used with sample preparation by double chemical fixation. Samples were pre-fixed in 2% glutaraldehyde in 0.5M phosphate buffer (pH 6) for 60 minutes and then washed twice during a 20 minute period in the same buffer. Post-fixing in 1% osmium tetraoxide in water for 60 minutes was followed by washing twice in distilled water. Samples were centrifuged, re-suspended in approximately 0.5 ml of water, frozen quickly in liquid nitrogen (-60°C, 10^{-2} torr) and sputter coated with gold. Samples were examined at magnifications up to 10,000.

3.3.8 Optical microscopy

Optical microscopy pictures of yeast cells were obtained using an Olympus BHS microscope connected to an Olympus PM10 camera. Fresh samples of the fermenting medium were examined at a magnification of 145.

3.3.9 Sugar analysis

The four main sugars, maltose, glucose, fructose and sucrose found in beer fermentations were quantified using test-combination kits (Boehringer Mannheim GmbH, Mannheim, Germany). The enzymatic methods for determination of sugars were based on the measurement of NADPH (nicotinamide-adenine dinucleotide

phosphate, reduced form) formation at 340 nm using a UNICAM 5625 UV-VIS Spectrometer.

3.3.10 Ethanol and other volatile compounds determination

3.3.10.1 Analysis of beer condensates

The analyses were performed with a Hewlett Packard 5890 series II gas chromatograph equipped with a HP 7673 automatic sampler, a flame ionisation detector (FID), and a HP 3396 series II integrator. The capillary column was a BP20 column (SGE Ltd., UK), 50m x 0.32 i.d., film thickness of 1 μm. Injector and detector were maintained at 200°C and 250°C respectively. Helium was used as the carrier gas at a flow rate of 1.5 $ml.min^{-1}$, and the injection size was 0.5 μl.

Condensate samples were diluted to 1:100 prior to ethanol analysis. The oven temperature was isothermal at 115°C for 5 minutes.

For the analysis of the other volatile compounds, condensate samples were injected directly onto the column without any dilution. The oven temperature was programmed as follows: 60°C for 8 min then raised to 200°C at $7°C.min^{-1}$, holding at 200°C for 5 min. 1-pentanol was used as an internal standard. A chromatogram of a beer condensate obtained using this method is displayed in Appendix B.

3.3.10.2 Analysis of beer

For the beer medium samples, a Hewlett Packard 5790 A series Gas Chromatograph equipped with a flame ionisation detector and interfaced with a LDC/Milton Roy CI-10B integrator was used. A 2.5 m long, 3 mm i.d. stainless steel column was packed with Chromosorb 101 (mesh size 80-100). The injector and detector were maintained at 200°C and 250°C respectively. Helium carrier gas flow was 40 $ml.min^{-1}$ and the injection size was 1 μl.

For ethanol analysis, beer samples were diluted to 1:100 prior to injection onto the packed column. The oven temperature was held at 150°C for 5 minutes.

For the analysis of the higher alcohols, beer samples were directly injected onto the column without dilution. The oven temperature was held at 150°C for 20 minutes. Butanol was used as an internal standard.

3.3.11 Identification of volatile compounds in beer condensates

These analyses were carried out in the Pharmacy and Pharmacology Department at the University of Bath. A Hewlett Packard 5890A Gas Chromatograph equipped with a Hewlett Packard Benchtop MS was used with a BP20 capillary column, 50 m x 0.32 mm i.d., 1.0 μm film thickness (SGE Ltd, England). The oven temperature was programmed at 60°C for 5 minutes, then raised to 230°C at 10°C.min^{-1}, holding at that temperature for 2 minutes. An analytical workstation software application was used to operate the instrument and view the results. Compounds were identified by comparison of their mass spectra with those contained in the National Institute of Standards and Technology (NIST) mass spectral database.

3.3.12 Identification of trace volatile compounds in beer

An attempt to identify the difference in beer volatile compositions between control and stripped fermentations was carried out. Development of a qualitative method was carried out using gas chromatography/mass spectrometry (GC/MS) interfaced with an ATD (Automatic Thermal Desorption) unit. The volatile compounds were concentrated on a suitable adsorbent by purge and trap before being thermally desorbed and injected into the GC.

3.3.12.1 Purge and Trap system

A beer sample (10 ml) was transferred into a 30 ml purge flask, which was then placed in a water bath at 40°C. The equilibration time was set up to 5 minutes, after which N_2 sparging (pre-filtered with activated charcoal) was initiated. Volatile components carried over in the gas stream were trapped onto the porous polymer Tenax TA (Alltech Associates Ltd, UK). Flow rates of the purge gas (N_2) were set at 25-30 ml.min^{-1} for the stripped beer and at 3-5 ml.min^{-1} for the control. A purge time of 2 hours was used for all samples.

3.3.12.2 Automatic Thermal Desorption

The thermal desorption apparatus (Perkin Elmer ATD 400) was interfaced with an Autosystem GC and controlled with a microprocessor. The trap containing the adsorbed volatiles was placed on the carousel of the ATD and was automatically loaded into the oven, heated rapidly to 250°C for 10 minutes. During the desorption time, the volatiles were purged with helium into the cold trap maintained at -30°C. When desorption was completed, the cold trap was rapidly heated to 250°C and held at that temperature for a further 10 minutes. The volatiles were transferred from the trap onto the GC column for subsequent separation and analysis by GC/MS.

3.3.12.3 Chromatographic conditions

A Perkin Elmer Autosystem GC equipped with a Perkin Elmer Q-Mass 910 Benchtop MS was used with a BP20 capillary column, 50 m x 0.32 mm i.d., 1.0 μm film thickness (SGE Ltd, England). The oven temperature was programmed at 60°C for 5 minutes, then raised to 230°C at 4°C.min^{-1}, holding at that temperature for 2 minutes. An analytical workstation software application was used to operate the instrument and view the results. Compounds were identified by comparison of their mass spectra with those in the NIST mass spectral database.

3.4 STRIPPING DURING BEER FERMENTATION

3.4.1 Preliminary experiments with standard OG (1050)

Preliminary experiments were carried out with beer wort of original gravity 1050, for the development of the experimental fermentation apparatus described in section 3.1, the investigation of the optimum fermentation parameters and the development of the various analytical methods. The fermentation parameters investigated were the formulation of beer wort, the fermentation temperature, the mixing and aeration technique, and the gas stripping conditions (i.e. gas flow rate).

3.4.1.1 Formulation of the wort

Wort composition greatly influences the speed of fermentation, the extent of fermentation, the amount of yeast produced, and the quality of the beer produced.

Fermentable carbohydrates, assimilable nitrogenous compounds (amino acids, purines and pyrimidines) and accessory food factors, such as ions, are essential wort constituents. In the present work, in order to have all the major nutrients in the final elevated-sugar beer wort, additional malt was added to the original beer kit recipe.

3.4.1.2 Fermentation temperature

Industrial lager fermentation, which generally uses bottom-fermenting yeasts, is usually carried out at temperatures between 8 and 12°C, while industrial ale fermentation, using generally top-fermenting yeasts, is usually carried out at higher temperature between 16 and 20°C. In the present work, a Home Brew kit for ale production was chosen with an arbitrary fermentation temperature of 16°C. The temperature itself was not a critical parameter in the present work, as long as it was kept constant over the different fermentations, for comparison purposes. A set of fermentations, using a wine yeast, was carried out at 22°C, due to the temperature requirement of the yeast. Fermentation temperature is an important parameter in industrial brewing, particularly in relation to the production of flavour compounds. A rise in fermentation temperature usually results in higher production of esters and fusel oil, which ultimately alters the organoleptic quality of the beer.

3.4.1.3 Mixing and aeration

In industrial brewing, the effect of agitating fermentation vessels includes both aeration and mixing of the medium. Both tend to accelerate fermentation, aeration by supplementing the dissolved oxygen to the wort, mixing by bringing yeast from the head, and yeast that has sedimented, into suspension. The overall action is to increase yeast crops and speed of fermentation. For some yeast strains agitation influences beer flavour by increasing the level of diacetyl and esters. It can also influence the shape of the yeast cells, by elongating them.

In the present work, different agitation techniques, including mixing the medium by hand once a day before sampling, or continuous mechanical stirring were tried. The most successful technique in terms of effectiveness and ease of use was the mechanical circulation of the medium using a peristaltic pump.

Aeration of wort, as described in section 3.1, was carried out before pitching, once the medium was cooled down to 16°C, by injecting air through the ring sparger until 44% saturation.

3.4.1.4 CO_2 flow rate and foaming

A CO_2 flow rate, as high as 5 $L.min^{-1}$ was chosen to achieve the desired stripping efficiency, in order to keep medium ethanol under a minimum value. However, without the use of an antifoam, this high flow rate resulted in over-foaming of the beer medium, and ultimately loss of the medium and termination of the fermentation run. Foaming was proportional to the flow rate. At low flow rates (such as 0.5 $L.min^{-1}$), the stripping rate was insignificant, resulting in a similar fermentation pattern between control and stripped fermentations. Figure 3–4 and Figure 3–5 show the ethanol production curve and the changes in the fermentation rate (measured in changes in specific gravity) in both control and stripped fermentations with an original gravity of 1050. Stripping had only little effect on the ethanol production or the specific gravity. Therefore, to enable efficient stripping, the gas flow rate was set up at $5L.min^{-1}$, and an antifoam was added in the fermentation medium before stripping was initiated. To enable true comparison, the antifoam was also added to the control fermentations.

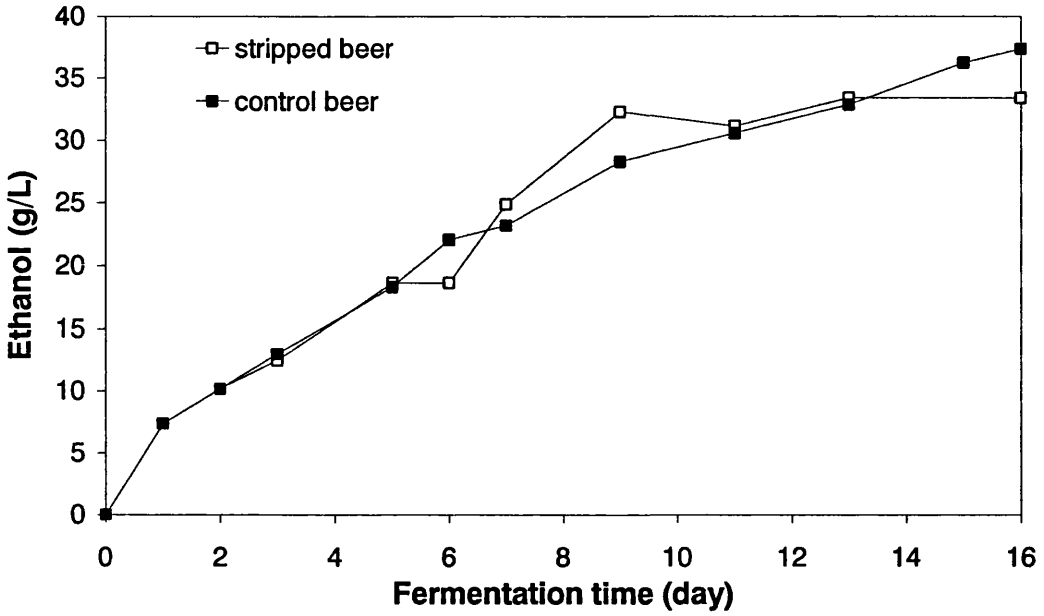

Figure 3–4: Effect of a small CO_2 flow rate of 1.8 l.min^{-1} on ethanol production during fermentations carried out with an original gravity of 1050.

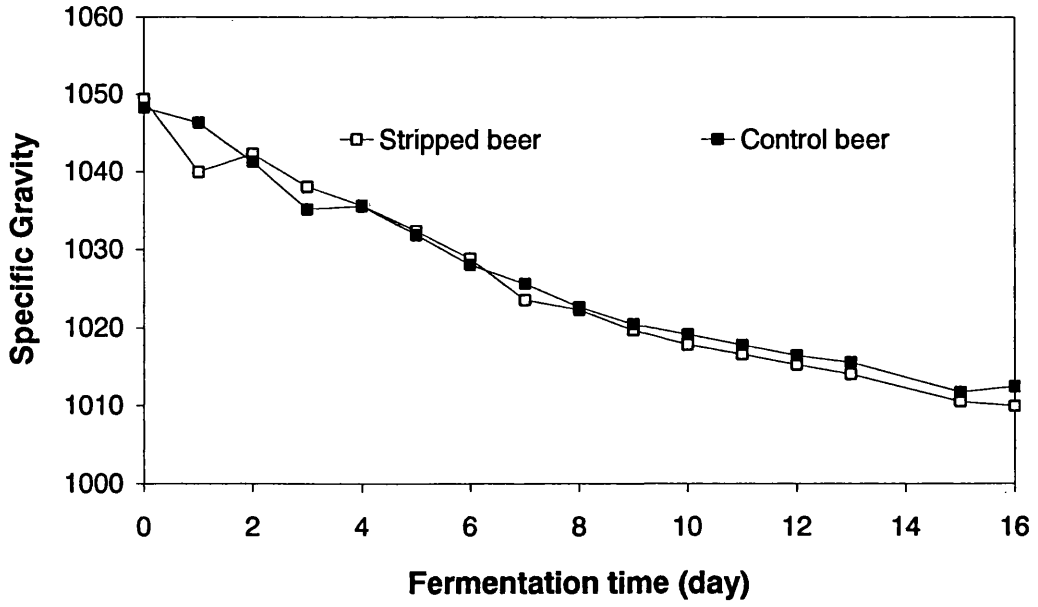

Figure 3–5: Effect of a small CO_2 flow rate of 1.8 L.min^{-1} on the fermentation rate during fermentation carried out with an original gravity of 1050.

3.4.2 High gravity fermentations

The study of CO_2 stripping during beer fermentation was carried out with worts of very high original gravity of 1080 and 1100, in the bench-scale 10L fermenters, as described in section 3.1. Stripped fermentations were run alongside control fermentations, in which no gas circulation was employed. Duplicate fermentations were carried out for every set of fermentations. CO_2 stripping was generally operated continuously during the fermentation, but the effect of periodical (or intermittent) stripping, where CO_2 was injected for only 2 or 3 days, was also assessed. Standard fermentations with agitation, initial aeration of the beer wort, and continuous stripping were carried out with both the original gravity of 1080 and 1100. Fermentations with OG 1100 were also carried out without agitation of the medium, and without initial aeration of the medium.

3.5 SYNTHETIC STRIPPING EXPERIMENTS

Stripping of synthetic mixtures was carried out with the same experimental apparatus as the one described for the beer fermentations (section 3.1). Aqueous solutions of ethanol and other beer volatiles were prepared following the concentrations shown in Table 3-2. The volatile compounds chosen for the stripping experiments were based on the identification of the compounds found in condensates of stripped beer fermentations. Their qualitative analysis was performed using the method described in section 3.3.11. Their concentrations were based on typical concentrations found in finished beer (Hough *et al.*[1982]). The stripping time was set to 10 hours, and the gas flow rate was varied from 1 to 5 $L.min^{-1}$. The two condensation stages were the same as described for the fermentations experiments. The coil condenser operating around 0°C (±2°C) was connected in series to a dry ice/acetone trap, operating at approximately –40°C (±10°C). Analysis of the mixtures and condensates were performed using the methods described in 3.3.10.1.

Table 3-2: Volatile compounds concentrations of the synthetic mixtures used in the CO_2 stripping experiments.

	VOC concentration ($mg.L^{-1}$)		
	Low	**Medium**	**High**
Acetaldehyde	0.5	7.6	15.8
Ethyl acetate	10.2	42.5	88.6
Methanol	1.0	3.4	8.8
Isobutyl acetate	1.1	4.1	7.9
Propanol	11.2	35.6	77.1
Isobutanol	17.6	68.7	146.1
Isoamyl acetate	1.4	5.1	9.6
1-butanol	1.0	4.0	7.4
Isoamyl alcohol	46.6	173.3	356.8
Ethyl caproate	1.1	4.4	8.7
1-hexanol	1.2	4.5	8.7
Ethyl caprylate	1.1	4.2	6.4
Ethyl caprate	1.1	4.3	8.6
Furfuryl alcohol	1.6	5.5	11.6
2-phenylethyl acetate	1.0	5.4	14.0
2- phenyl ethanol	7.9	33.8	81.7

3.6 PERVAPORATION STUDIES

3.6.1 Pervaporation apparatus

The pervaporation experiments were carried out using a standard batch pervaporation rig as described in Figure 3–6. A membrane disc was clamped into a sealed stainless steel cell on a porous sintered support. The pervaporation cell was filled with a feed solution and placed upon a stirrer/hotplate. The feed solution was continuously stirred with a magnetic follower. The cell temperature was controlled and measured with a thermocouple and electronic temperature control system. A vacuum was created, by means of a vacuum pump, and the downstream pressure measured between the cell and the cold traps. The permeate was condensed and frozen using a cold trap containing liquid nitrogen.

Pervaporation experiments were carried out with 450 g of feed solution, maintained at 40.0 ± 0.5°C, and continuously stirred. The pressure of the permeate side was maintained below 2 mbar. Permeate was collected after two hours of operation. Each experiment was repeated three times and the results averaged. Two types of membrane were tested; a standard PDMS (Polydimethyl siloxane) membrane and a modified PDMS membrane supplied by GKSS (no detail on the composition of the membrane was provided by the supplier except that it contained long alkyl chains).

3.6.2 Feed solutions and analyses

The feed solutions were synthetic mixtures containing ethanol, isoamyl alcohol, isobutanol and propanol in water. Two mixtures were prepared: an equi-concentration mixture containing 0.5 $g.L^{-1}$ of each organic compound and an artificial beer condensate containing 100 $g.L^{-1}$ (12.6% v/v) ethanol, 20 $mg.L^{-1}$ propanol, 80 $mg.L^{-1}$ isobutanol and 400 $mg.L^{-1}$ isoamyl alcohol.

During the pervaporation experiments, the total flux was measured and the concentrations of the organic compounds in the feed and the permeate were analysed using the analytical method described in section 3.3.10.1 for condensate analysis.

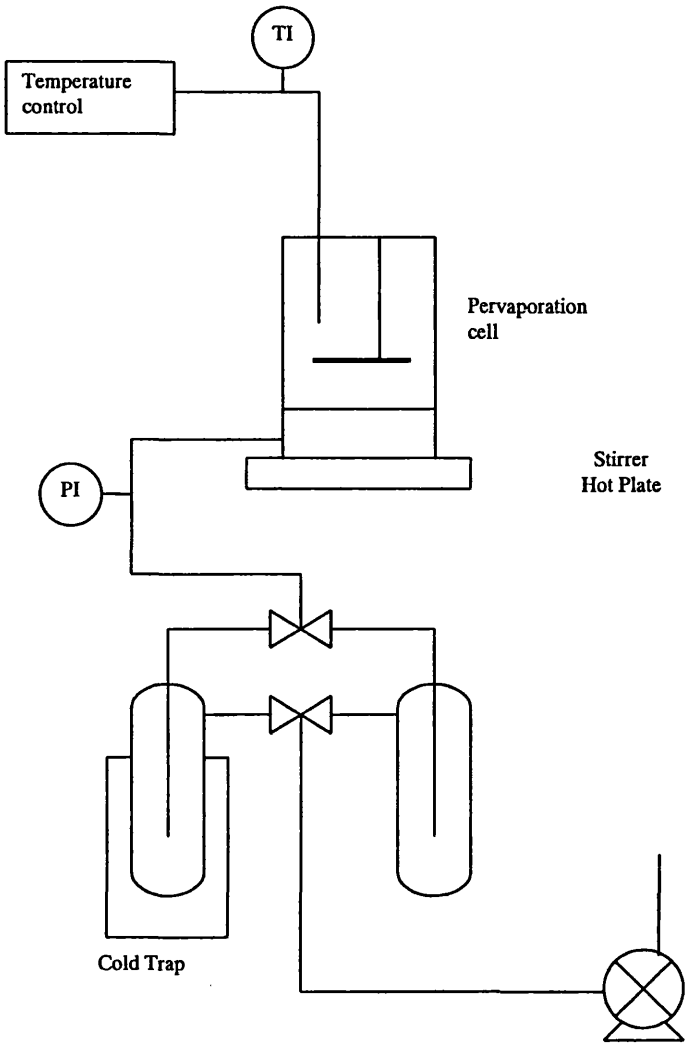

Figure 3–6: Pervaporation apparatus (reproduced from Bennet [1996]).

CHAPTER 4 - PREDICTING THE VOLATILITY OF BEER FLAVOUR COMPOUNDS

4.1 INTRODUCTION

Gas stripping is a process driven by vapour-liquid equilibrium (VLE), which represents the limiting condition for any gas-liquid contact. The higher the volatility of a compound, the greater will be the driving force and hence the removal of this compound by gas stripping. If gas stripping is applied to a multi-component system such as a fermentation broth, it could ultimately change the volatile compound ratio in the liquid medium. A knowledge of the volatility of beer flavour compounds would, therefore, be a useful tool to understand the effect of gas stripping on the chemical balance of a liquid system such as beer. After outlining the basic principles of VLE, this chapter presents an estimation of selected beer compound volatilities. Selection of the volatile compounds was based on their individual occurrence in collected condensates of a fermentation carried out with an original gravity of 1100. Experimental data arising from stripping experiments on synthetic mixtures containing the selected flavour compounds were compared with predicted values.

4.2 BASIC PRINCIPLES OF VLE

At low pressures (approximately below 2 bars) where the ideal gas law applies to the vapour phase and the liquid phase properties are independent of pressure, the vapour liquid equilibrium of an aqueous solution can be written as:

$$y_i P = f^\circ_i \gamma x_i \qquad \textit{Equation 4-1}$$

Where, x_i: mole fraction of component i in the liquid phase
y_i: mole fraction of component i in the vapour phase
P: total pressure
f_i°: fugacity of compound i
γ_i: activity coefficient of component i

For a pure liquid compound, its fugacity f°_i is the same as its vapour pressure P°_i (atm) under normal stripping pressures, and Equation 4-1 becomes:

$$y_i P = P^\circ_i \gamma x_i \qquad \textit{Equation 4-2}$$

The activity coefficient γ_i is a parameter which represents the deviation from ideality of a solution composed of several compounds different in nature. This parameter is

unaffected by small changes in temperature but is strongly concentration dependant. When γ_i is greater than 1, the system is said to show positive deviations from ideality, when less, negative deviation. The activity coefficient γ_i varies from 1 for x_i equal to unity to its highest value γ^∞ (activity coefficient at infinite dilution) for x close to zero. The two limiting cases for a binary system such as the ethanol-water system described in Figure 4–2 are therefore:

1) If the liquid phase behaves like an ideal solution (case of a pure liquid) with no interaction between the dissolved material and the solvent water, the VLE follows Raoult's Law, in which the activity coefficient γ_i is equal to 1 and is described by:

$$yi = \frac{P_i^\circ}{P} xi \qquad \textit{Equation 4-3}$$

2) In dilute solutions, where the activity coefficient γ_i is approximately constant and equal to γ_i^∞, the infinite-dilution activity coefficient, Equation 4-1 is linear under a fixed pressure. This linear relation can be represented using an equilibrium constant K^∞ at the limit of infinite dilution as follows:

$$y_i = K^\infty x_i \qquad \textit{Equation 4-4}$$

$$\text{where } K^\infty = \frac{P_i^\circ \gamma^\infty}{P}$$

K^∞ value is a measure of the volatility of an organic compound. The larger the K^∞ value, the greater will be the equilibrium concentration of an organic compound in the gas phase. Thus, organic compounds with large K^∞ value are more easily removed by gas stripping.

K^∞ values are in fact dimensionless Henry's Law constants, which are more commonly used for example in pollution control calculations. Most of the work on estimation of Henry's Law Constants has been carried out in the field of organic pollutants in water (Hwang *et al.* [1992]; Shiu and Mackay [1997]; Hovorka and Dohnal [1997]), where gas stripping is a well established method of decontamination.

Henry's Law constants have been defined in many combinations of units and therefore can be a source of confusion. However the most common definitions are probably $H=p_i/x_i$ in atm and $H=p_i/c_i$ in $atm.m^3.mol^{-1}$. Henry's constants are usually defined for binary systems where interaction occurs between an organic solute and the solvent water, while neglecting any weak interactions occurring among the organic solutes.

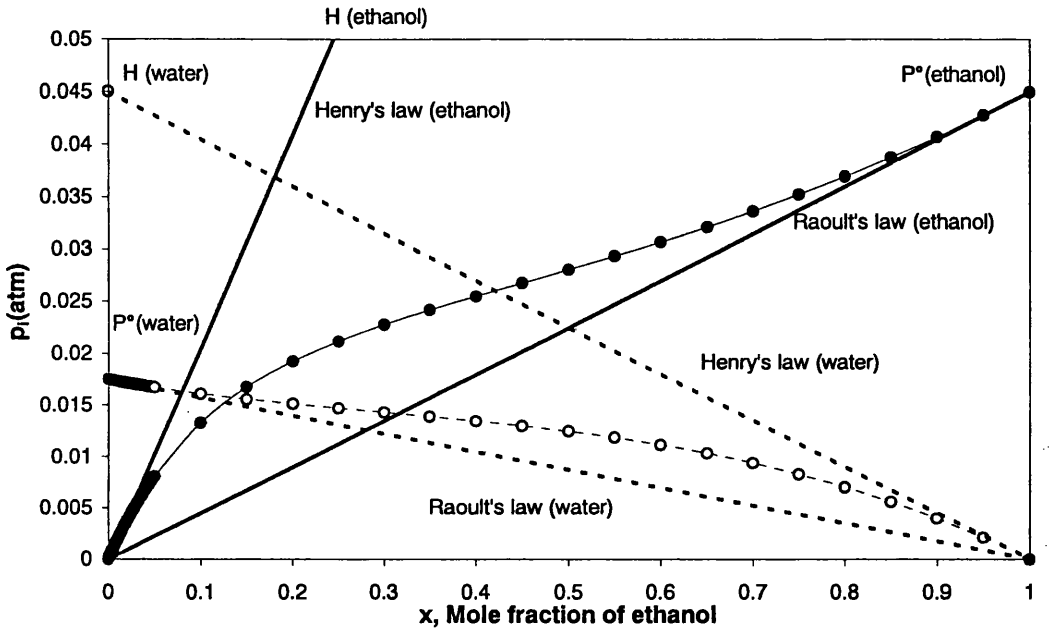

Figure 4–1: Representation of water-ethanol vapour-liquid equilibrium at 16°C. p° and γ^{∞} were computed using the Antoine Equation and the UNIFAC method respectively.

4.3 PREDICTION OF K^{∞} VALUE

Only volatile organic compounds detected in natural condensate, by the analytical method described in Chapter 3, were investigated. Natural condensate was the fraction collected from CO_2 stripping of beer fermentations carried out at high original gravities, as studied in Chapter 5. Some of the chemical and physical properties of the selected beer volatile compounds are presented in Table 4-1. In addition, molecular structures of those compounds are presented in Appendix E.

Ethyl caproate, ethyl caprylate and ethyl caprate, which are esters containing long hydrophobic alkyl chains (C_6, C_8 and C_{10} respectively), and 2-phenylethyl acetate

containing a benzene ring, are insoluble in water. However, at the concentration found in beer, they are soluble due to the relatively high concentration of ethanol in the alcoholic beverage (between 2 and 10%v/v).

Table 4-1: Physical and chemical properties of selected beer volatile compounds.

Volatile compound	Chemical Formulae	MW ($g.moL^{-1}$)	Bp °C 760mmHg	Density ($g.mL^{-1}$) 20°C	Solubility ($g.mL^{-1}$) 25°C
Ethanol	C_2H_6O	46	78	0.785	misc.
Acetaldehyde	C_2H_4O	44	21	0.788	misc.
Acetone	C_3H_6O	58	56	0.790	misc.
Methanol	CH_4O	32	64.6	0.790	misc.
Ethyl acetate	$C_4H_8O_2$	88	77	0.900	0.09
Propanol	C_3H_8O	60	97	0.804	misc.
Isobutanol	$C_4H_{10}O$	74	106	0.801	0.04
Isobutyl acetate	$C_4H_{10}O$	116	117	0.870	0.005
1-Butanol	$C_6H_{12}O_2$	74	117	0.810	0.07
Isoamyl alcohol	$C_5H_{12}O$	88	131.5	0.809	0.03
Isoamyl acetate	$C_7H_{14}O_2$	130	142	0.876	0.002
1-Hexanol	$C_6H_{14}O$	102	157.6	0.814	slightly sol.
Ethyl caproate (ethyl hexanoate)	$C_8H_{16}O_2$	144	168	0.873	insol.
Furfuryl alcohol	$C_5H_6O_2$	98	171	1.132	misc.
Ethyl caprylate (ethyl octanoate)	$C_{10}H_{20}O_2$	172	208.5	0.867	insol.
2-Phenyl ethanol	$C_8H_{10}O$	122	216	1.020	
2-Phenylethyl acetate	$C_{10}H_{12}O_2$	164	226	1.031	insol.
Ethyl caprate (ethyl decanoate)	$C_{12}H_{24}O_2$	200	241.5	0.865	insol.

4.3.1 Estimation of theoretical K^{∞} values for beer volatile organic compounds.

The task of estimating a Henry's Law constant (or K^{∞} value) for a volatile compound can be divided into the estimation of its vapour pressure P°, and its infinite-dilution activity coefficient γ^{∞} at the equilibrium temperature T. Methods for estimation of P° and γ^{∞} for binary systems are well reviewed by Hwang *et al.* [1992].

Volatile concentrations in beer range from 1 or 2 $mg.L^{-1}$ (i.e. isoamyl acetate) to about 500 $mg.L^{-1}$ (i.e. isoamyl alcohol). The system can be considered dilute, and Henry's Law (Equation 4.4) can be applied to predict each volatile compound's partial pressure. K^{∞} values for some of the beer volatile compounds were found in the databank published by Hwang *et al.* [1992], for temperatures of 25° and 100°C. K^{∞} being a function of the exponential of the temperature (ln K is a function of 1/T), K^{∞} values at 16°C were extrapolated from K^{∞} values at 25°C and 100°C. However, K^{∞} values were estimated for binary systems containing water and one dilute volatile organic compound. This is clearly not the case in beer since the beverage contains other organic compounds, such as ethanol and sugars at relatively high concentration, which can potentially affect the volatility of the compounds.

An attempt in predicting the VLE of such a complex system was carried out. For multi-component systems, the Wilson or UNIQUAC methods can be used to predict the infinite-dilution activity coefficient, if each two-component parameter (interaction between solutes) is available from the literature. Unfortunately, for the present study it was not possible to find published experimental data for all pairs of compounds. The UNIFAC (UNIQUAC functional group activity coefficient) method, group contribution method based on molecular structure, was therefore used for the calculation of infinite-dilution activity coefficient, γ^{∞}. The method was applied to binary (for comparison with Hwang Henry's Law constants), ternary (water-ethanol-VOC) and quaternary (water-ethanol-glucose-VOC) systems. A UNIFAC program written in and running under MS-DOS was provided by Lipnizki [1999].

Ethanol concentration in beer varies from approximately 2 to 10% v/v, and thus can not be considered dilute. Within that range of concentrations, the activity coefficient

of ethanol varies and the VLE curve is then not linear. However, when necessary, partial pressure of ethanol was calculated for defined medium concentrations, using the UNIFAC program for the calculation of γ at the system temperature.

The vapour pressure of a pure liquid component was calculated using the Antoine equation, as follows:

$$\log(P^{\circ}) = A - \frac{B}{T + C}(atm)$$

where, constants A, B and C were found in the literature (Coulson and Richardson [1983], Hirata et al. [1976], Gmehling and Onken [1977]).

Vapour pressure and infinite dilution activity coefficient calculated using the UNIFAC method for binary (water and one volatile organic compound), ternary (water, ethanol at 5% v/v and one volatile organic compound) and quaternary (water, ethanol at 5% v/v, glucose at 80 g/L and one volatile organic compound) systems at 16°C, are presented in Table 4-2, along with binary Henry' Law constants, found in the literature (Hwang *et al.* [1992]) and corrected for temperature.

The compounds studied in Table 4-2 can be classified into three categories, alcohols, aldehydes and esters. There are other classes of aroma compounds in beer as described in Chapter 2, however, only selected compounds, as mentioned above, were investigated. As shown by their relatively high K^{∞} value, esters are highly volatile in aqueous solution. This relatively high volatility compared to alcohols is mainly due to their high infinite dilution activity coefficient, related to their hydrophobic character. The longer the alkyl chain of the molecule (such as in ethyl hexanoate, octanoate and decanoate), the higher the hydrophobicity and the infinite dilution activity coefficient are. Aldehydes and alcohols, at the contrary, are more hydrophilic, due to the relatively higher polarity of the oxygen atom of the alcohol and aldehyde function. For that reason, their infinite dilution activity coefficient, and their K^{∞} value are lower than for esters. The same comment can be made concerning the alkyl chain in the alcohols. From methanol (C_1) to hexanol (C_6), the hydrophilicity decreases and consequently the infinite-dilution activity coefficient increases.

Table 4-2: Estimated K^{∞} values of selected beer volatile organic compounds in dilute aqueous solutions at atmospheric pressure.

Compound	p°(atm)[I]	γ^{∞} (UNIFAC)[II]			H[III]	K^{∞} value (UNIFAC)[IV]		
	16C	Binary[i]	ternary[ii]	Quaternary[iii]	Binary[i]	Binary[i]	ternary[ii]	Quaternary[iii]
Acetaldehyde	8.4E-01[1]	4.76	4.44	3.71	3.159	3.975	3.709	3.100
Ethyl acetate	8.1E-02[1]	66.47	58.48	52.39	0.952	5.361	4.717	4.226
Methanol	1.0E-01[1]	1.571	1.526	1.419	0.173	0.160	0.155	0.145
Isobutyl acetate	1.3E-02[1]	631.70	518.10	434.9	11.960[c]	8.231	6.751	5.667
Propanol	1.4E-02[1]	13.42	12.03	10.67	0.200	0.191	0.172	0.152
Isobutanol	8.9E-03[3]	40.89	35.37	30.37	0.346	0.362	0.313	0.269
Isoamyl acetate	4.5E-03[2]	2024	1603	1302		9.182	7.272	5.906
1-butanol	3.9E-03[1]	40.89	35.38	30.37	0.371	0.161	0.139	0.119
Isoamyl alcohol	2.1E-03[2]	127.40	106.30	88.37	0.250[d]	0.272	0.227	0.189
Ethyl caproate	1.9E-03[2a]	9764	7424	5926		18.277	13.897	11.093
1-hexanol	4.9E-04[1]	403.00	325.00	261.3	0.350	0.198	0.160	0.129
Ethyl caprylate	1.9E-03[2a]	108600	77050	57540		203.283	144.226	107.707
Ethyl caprate	1.9E-03[2a]	1242000	823200	57480		2325	1541	107.594
Furfuryl alcohol	4.1E-04[2]	94.62[b]	82.59[b]	26.16[b]	0.081[e]	0.039	0.034	0.011
2-phenylethyl acetate	1.6E-05[2]	76150	53850	3618		1.198	0.847	0.057
2-phenyl alcohol	8.4E-06[2]	3240	2377	1691	0.016	0.027	0.020	0.014

[I] Vapor pressure (atm) calculated from the Antoine Equation, at 16°C. [II] γ^{∞} estimated using UNIFAC. [III] Corrected Henry's Law Constant from Hwang *et al.* [1992] at 16°C. [IV] K^{∞} value predicted using calculated Vapor pressure and estimated $\gamma^{\infty}$from UNIFAC. [1]Antoine constants from Coulson and Richardson [1983]. [2] Antoine constants from Hirata *et al.* [1976]. [3] Antoine constants from Gmehling and Onken [1977]. [i] Aqueous system containing dilute ethanol and VOC. [ii] Aqueous system containing 5% v/v ethanol and dilute VOC. [iii] Aqueous system containing 5% v/v ethanol, 80 $g.L^{-1}$ glucose and dilute VOC. [a] vapour pressure of ethyl isocaproate. [b] γ^{∞} for furfural. [c] Corrected Henry's Law constant (Hwang *et al.* [1992] for n-butyl acetate, not isobutyl acetate. [d] Corrected Henry's Law constant (Hwang et al.[1992]) for 1-pentanol, not for isoamyl alcohol. [e] Corrected Henry's Law constant (Hwang *et al.*, [1992]) for furfural not for furfuryl alcohol.

4.3.2 Effect of other beer components on K^{∞} values

4.3.2.1 Effect of ethanol concentration

During fermentation of high gravity beer medium, ethanol concentrations varied greatly from zero at the start of the fermentation to approximately 8% v/v at the end of the fermentation (Chapter 5). When stripping was activated during the fermentation, the maximum ethanol concentration reached in the medium was 6.5% v/v. From day 3 (start of stripping) to day 16, ethanol in the stripped medium varied from approximately 3 to 6.5% v/v. As shown in Table 4-2 for ternary systems (water, ethanol 5% v/v and one VOC), γ^{∞} for all of the compounds decreases when ethanol is present in the medium.

Figure 4–2 shows the decrease in infinite-dilution activity coefficient for selected compounds, when ethanol concentrations vary from 2 to 10% v/v. The decrease in γ^{∞} leads to a decrease in the K^{∞} value and consequently in the volatility of the beer volatile compounds.

4.3.2.2 Effect of glucose concentration

Fermentation medium typically contains high levels of soluble sugars, such as glucose, fructose, sucrose and maltose. Sugar concentrations are high at the beginning of the fermentation but reduced during the fermentation due to yeast metabolism. In standard brewing (standard original gravity), where fermentation goes to completion, only residual sugars remain in the beer medium at the end of the fermentation. In high-gravity fermentation, sugars can be present in relatively large quantities at the end of the fermentation, due mainly to ethanol inhibition. The presence of sugars in the medium is likely to affect the volatility of beer volatile compounds, and ultimately the removal by gas stripping of these compounds from fermenting media.

Using the UNIFAC program, $\gamma^{\infty}$was calculated for aqueous system containing 5% of ethanol and 80g.L^{-1} of glucose. (Glucose was the only sugar available in the UNIFAC program databank). As shown in Table 4-2, γ^{∞} of all beer volatile compounds in this quaternary system was decreased compared to γ^{∞} calculated for ternary system containing only water and 5% ethanol.

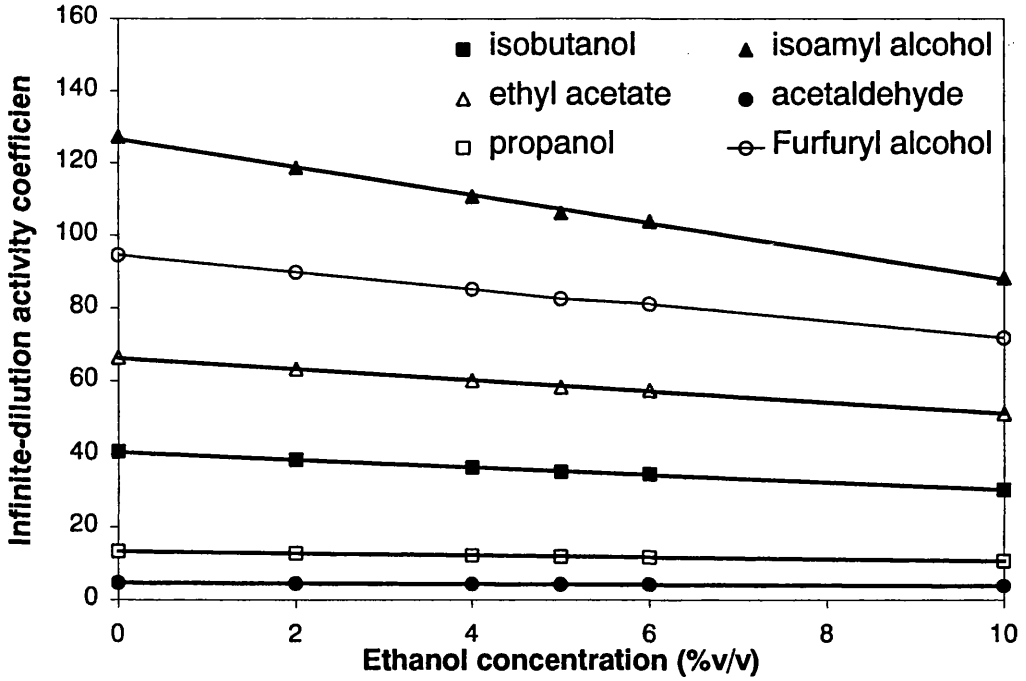

Figure 4–2: Influence of ethanol concentration on the infinite-dilution activity coefficient of the main beer volatile compounds (γ^{∞} computed with the UNIFAC program)

4.4 EXPERIMENTAL DATA USING SYNTHETIC MIXTURES

Gas stripping experiments were carried out using the laboratory scale fermenters, as described in Chapter 3. The stripping system was not designed to fully recover the extracted volatiles. However, experiments carried out with synthetic mixtures were aimed to help the understanding of CO_2 stripping with real fermentation medium. The experimental data was compared with theoretical data from Table 4-2 to evaluate the efficiency of the gas stripping apparatus.

The synthetic mixtures were composed of ethanol and 16 other beer volatile compounds, identified previously in natural condensates by GC/MS (see Chapter 5). Their concentrations were typical concentrations found in finished beer (Hough *et al.* [1982]), and are indicated in Table 3-2 (Chapter 3). Experiments were carried out with combinations of three different initial concentrations for all the compounds. Gaseous CO_2 was supplied from a gas cylinder. The stripping time was set to 10 hours so that the concentration of the different volatile compounds would not change

dramatically during the stripping time and that the volume of collected condensate would not significantly decrease the total medium volume. Two condensation stages as described in Chapter 3 were used: a coil condenser operating at 0°C (±2°C) was connected in series to a dry ice/acetone trap, operating at approximately –40°C (±10°C).

4.4.1 Efficiency of the condensation system

Theoretical ethanol vapour mole fractions were calculated using Equation 4-2, where the activity coefficients for various ethanol liquid mole fractions were estimated using the UNIFAC program. Theoretical water vapour mole fraction was calculated using Raoult's Law (Equation 4-3) as water liquid mole fraction tended to 1.

Percentage recovery, calculated as the ratio between experimental vapour mole fraction and theoretical vapour mole fraction, was 24% for ethanol and 74% for water (Table 4-3). Experimental ethanol and water compositions in the vapour phase (from condensed fraction) were always lower than the theoretical calculations (using UNIFAC). Three hypothesis were drawn from this:

1) Equilibrium was not reached between the gas and liquid phase, due to insufficient contact time between the gas and liquid phase and/or to small gas-liquid surface/volume ratio.
2) The condensation system (coil condenser and cold trap) was not efficient enough to recover the totality of the extracted volatile compounds.
3) There was a leak in the system.

If equilibrium was not reached completely, the ratio between calculated and measured ethanol mole fraction would be expected to be higher than that of water, as ethanol is more volatile than water. The contrary was found. So, the second hypothesis of partial condensation was retained and the first hypothesis was rejected. The more volatile a compound, the easier it is stripped from the liquid phase, but the greater the condensation requirement. Although the apparatus was checked for leaks before the experiments were performed, the possibility of a leak in the system was not totally rejected. However, partial condensation was more likely to be the major contributor of the system inefficiency.

Partial condensation was also used to interpret the experiments carried out at different CO_2 flow rates (Table 4-4). At 1L.min^{-1}, 98% of the water and 52% of ethanol were condensed compared to 74% and 24% at 5L.min^{-1}. Lowering the CO_2 flow rate resulted in a higher condensation efficiency.

Table 4-3: Calculation of the recovery (%) of water and ethanol by the condensation system during CO_2 stripping of an aqueous solution containing 5% ethanol and dilute VOCs at 16°C (5 L.min^{-1} of CO_2).

	Water	**Ethanol 5% v/v**
x_i	1	0.0151
$P_i°$	0.0175	0.0449
γ_i	1	4.236
$y_i(1)$	0.0175	0.0029
x_{cond}	0.95	0.05
$y_i(2)$	0.0130	0.0007
Recovery (%)	74	24

x_b, measured medium liquid mole fraction

$P_i°$, vapour pressure (atm).

γ_b estimated activity coefficient (UNIFAC)

$y_i(1)$, theoretical vapour mole fraction from estimated $p°$ and γ

x_{cond}, measured condensate liquid mole fraction

$y_i(2)$, vapour mole fraction in the CO_2 phase estimated from x_{cond} and the volume of CO_2

Recovery (%) = $y_i(2).100/ y_i(1)$

Table 4-4: Effect of the CO_2 flow rate on the recovery (%) of water and ethanol by the condensation system.

	CO_2 flow rate (L.min^{-1})		
	5	**3**	**1**
Water	74	84	98
Ethanol	24	30	52

4.4.2 Comparison of beer compounds theoretical volatility with experimental data

Experimental vapour mole fractions (estimated from measured condensate concentrations and CO_2 flow rate) of the different volatile compounds were plotted against the initial mole fraction for the three initial concentrations used. The slope of the linear fit was compared with the K^∞ value and corrected Henry's Law constant (Hwang *et al.* [1992]) for each compound (Table 4-5). As expected, theoretical K^∞ values for all of the compounds were larger than the experimental slope, due to partial condensation. However, the experimental order of volatility of the different

compounds, was very similar to the theoretical order of volatility, i.e. alcohols and aldehydes were less volatile than esters. The percentage recovery calculated as a ratio between the slope and theoretical K^{∞} or between the slope and theoretical Henry's Law constant (Hwang *et al.* [1992]), shows that the most volatile compounds were the least successfully recovered, as would be expected. Between 13 and 45% w/w of the volatile alcohols were condensed whereas only between 0.2 and 9.3% w/w of esters and aldehydes were condensed.

Table 4-5: Comparison between the experimental slope (a) with K^{∞} value and corrected Henry's Law constant (H). Percentage recovery (%) expressed as the ratio between a and K^{∞} or between a and H.

	a Exp. Slope	K^{∞} value (Ethanol 5%v/v)	Corrected H	% Recovery	
				a/K^{∞}	a/H
Furfuryl alcohol	0.004	0.034	0.081	13.0	5.5
2-phenyl ethanol	0.004	0.020	0.016	21.0	25.7
Methanol	0.021	0.155	0.173	13.7	12.3
Propanol	0.044	0.172	0.200	25.6	22.0
1-butanol	0.049	0.139	0.371	35.4	13.3
Isoamyl alcohol	0.065	0.227	0.250	28.5	25.8
Isobutanol	0.068	0.313	0.346	21.7	19.6
1-hexanol	0.072	0.160	0.350	45.0	20.5
2-phenylethyl acetate	0.075	0.847		3.1	
Acetaldehyde	0.080	3.709	3.159	1.3	1.6
Ethyl acetate	0.169	4.717	0.952	3.6	17.8
Isobutyl acetate	0.335	6.751	11.960	5.0	2.8
Ethyl caproate	0.523	13.897		3.8	
Isoamyl acetate	0.678	7.272		9.3	
Ethyl caprylate	1.903	144.226		1.3	
Ethyl caprate	2.910	1540.911		0.2	

4.4.3 Condensation stages

4.4.3.1 Air stripping

Preliminary air stripping experiments were carried out with a mixture of only five volatile compounds including ethanol, prepared following the concentrations indicated in Table 4-6. Continuous stripping at 0.34 $L.air.min^{-1}.L^{-1}$ medium was carried out for 200 hours, and extracted volatile compounds were condensed using a coil condenser only, maintained at approximately –4°C.

As discussed previously, only partial condensation of the different compounds was achieved, using the condensation system and temperature. A mass balance for the extracted ethanol mass (calculated from the remaining ethanol mass at the end of the 200 hours stripping) and the total condensed ethanol mass showed that some of the extracted ethanol was not recovered. The same was observed for the other volatile compounds. Table 4-6 summarises the air stripping experiments, and shows the percentage of extracted (stripped) volatile compound mass compared to the recovered (condensed) compound mass. As expected, the order of volatility of the different volatile compounds follows the theoretical order given in Table 4-2. Only 0.5% of ethyl acetate, which is the most volatile compound out the five, was condensed, whereas 21% of ethanol, which is the least volatile, was recovered. It was also observed during this experiment that 99% of ethyl acetate was extracted after 65 hours of stripping, whereas a relatively large proportion of the other volatiles remained in the medium at the same time.

Table 4-6: Air stripping of synthetic mixtures of volatile organic compounds. Percentage of volatile compounds extracted and recovered by condensation (-4°C) after 200 hours of air stripping.

	Initial Concentration	% Extracted	% Recovered
Ethanol	42 $g.L^{-1}$	39	21
Propanol	70 $mg.L^{-1}$	47	18
Isoamyl alcohol	207 $mg.L^{-1}$	65	17
Isobutanol	122 $mg.L^{-1}$	66	14
Ethyl acetate	21 $mg.L^{-1}$	99	0.50

4.4.3.2 CO_2 stripping

Based on the results from Table 4-3, total ethanol recovery was only about 24% when using both condensation units, at 0°C and -40°C. Table 4-7 shows that out of the total 24% of condensed ethanol, 33% was condensed by the coil condenser at 0°C, and 67% by the trap at -40°C. All other volatile compounds were similarly partially condensed, and relative quantities recovered by the two condensers varied depending on the degree of volatility of the compound. Therefore, two different fractions in terms of ratio of volatile organic compounds were collected by the two condensation stages. The 0°C condensate contained mainly water, ethanol and the majority of the least volatile compounds (such as furfuryl alcohol and 2-phenyl ethanol) whereas the trap contained most of the very volatile esters.

Table 4-7: Percentage of volatile compounds, in % w/w of the total fraction, recovered by each condensation unit (coil condenser at 0 °C and trap at -40 °C).

	Total fraction %w/w (Table 4-5)	**% recovered by the coil condenser (0°C)**	**% recovered by the trap (-40°C)**
Furfuryl alcohol	13.0	97	3
2-phenyl ethanol	21.0	89	11
Methanol	13.7	73	27
Propanol	25.6	41	59
2-phenylethyl acetate	3.1	37	63
Ethanol	24	33	67
1-butanol	35.4	31	69
1-hexanol	45.0	26	74
Isoamyl alcohol	28.5	24	76
Isobutanol	21.7	22	78
Acetaldehyde	1.3	16	84
Ethyl acetate	3.6	2.3	98
Isobutyl acetate	5.0	0.30	100
Ethyl caprylate	1.3	0.04	100
Isoamyl acetate	9.3	0.04	100
Ethyl caproate	3.8	0	100
Ethyl caprate	0.2	0	100

4.4.4 Conclusion

Estimation of the relative volatility of the different beer condensate volatiles using UNIFAC provides a method of predicting the relative rate of extraction of these compounds by gas stripping. The theoretical relative volatilities of the various compounds agreed with the experimental data, despite the variation in absolute values due to partial condensation. Although the recovery of the volatile compounds by the condensation units was incomplete, the estimation of the loss (by comparison between theoretical data and experimental data) can be a useful tool. It could indeed provide a mean of predicting the total production of volatile compounds, when gas stripping is applied to real fermentation media.

CHAPTER 5 - CO_2 STRIPPING DURING HIGH GRAVITY FERMENTATION

5.1 RESULTS

The primary objective of the work undertaken was to investigate the feasibility of CO_2 stripping as a technique for ethanol removal from high-gravity beer fermentations. In addition, an attempt to understand the other changes occurring during stripped fermentations was made, as CO_2 stripping does not have the sole effect of removing ethanol from the fermentation medium. A series of experiments were carried out to determine the effect of stripping on the physical and chemical properties of high-gravity beer fermentations. Results concerning the fermentation experiments carried out in the present study are presented in this section.

5.1.1 Fermentations details

5.1.1.1 Investigated fermentation parameters

The present study is a follow-up to previous work carried out in this laboratory by Huxtable [1993] on CO_2 stripping of cider fermentations, using recirculation of naturally produced CO_2. In the present work, CO_2 stripping was carried out with high-gravity beer fermentation in bench-scale 10L fermenters, a change to the 35L tower fermenters used by Huxtable [1993]. The volume of the beer fermentation was not sufficient to self generate CO_2, rather an external source was used.

Stripped fermentations were run alongside control fermentations, in which no gas circulation was employed. Stripping was initiated after 3 days of fermentation in order to allow undisturbed exponential yeast growth. As well as varying the original gravity of the initial wort, the effect of mechanical agitation during the whole course of the fermentation and the effect of initial aeration of the beer wort was assessed. CO_2 stripping was generally operated continuously during the fermentation, but the effect of intermittent stripping was also evaluated.

Thorough analysis of changes during fermentation of high gravity wort was achieved through follow-up of the fermentation products such as ethanol and other beer volatile compounds, the sugar uptake, the rate of fermentation (measurement of specific gravity), the acidity (measurement of pH), and the yeast morphology and physiology.

5.1.1.2 Changes in beer medium volume during fermentation.

Changes in beer volume during fermentation occurred for both the stripped and control beers. Sampling for analysis caused an average volume loss of approximately 10% in both the stripped and control beers. In addition, when stripped, water along with ethanol and other volatile compounds were extracted from the fermentation medium. Approximately 10% of the initial beer volume was collected as condensate. Moreover as discussed in Chapter 4, not all of the extracted volatile compounds were captured by the condensation units. Therefore, another volume loss not accounted for in the results presented in this chapter occurred for the stripped beer. Finally, the production of gaseous carbon dioxide contributed also to a loss of fermentation volume in both control and stripped media. Table 5-1 summarises the different volume losses occurring in both control and stripped fermentations.

Table 5-1: Examination of volume losses during the fermentation of both control and stripped medium.

Reason for volume loss	**Occurrence**	**Loss: % of initial fermentation volume**
Sampling	Control and stripped	10%
Collected Condensate	Stripped	10%
Stripped compounds but not condensed	Stripped	Not measured
CO_2 production	Stripped and control	Not measured

5.1.2 Examination of control fermentations

Before analysing the effect of CO_2 stripping of high gravity beer fermentation, a detailed examination of the control fermentations, where no gas circulation was carried out, is presented. High-gravity media (OG 1080 and 1100) were produced by increasing the amount of added sugars to a normal gravity (OG 1050) medium, which led to high concentrations of sugars in the medium.

Data for the control fermentations are summarised in Table 5-2, which also presents calculated fermentation efficiency from sugar and ethanol measurements on day 14. All the fermentations were run for at least 14 days, when all major measurements (sugar and ethanol) were carried out. However, the fermentations were usually prolonged for an extra two days until day 16. Final medium ethanol concentration (in g per litre of final volume) was measured on day 16 to allow the ethanol plateau to be established. It is a direct measurement, which does not take into consideration the loss of ethanol by sampling and the change of volume. Both ethanol production and sugar uptake on day 14 (in g per L of initial volume) included ethanol and sugar losses through sampling. Details of the results for the different fermentations are presented in the following sections.

Percentage fermentation efficiency was calculated from sugar and ethanol measurements on day 14 as follows:

$$\%\ fermentation\ efficiency = \frac{ethanol_{meas}}{ethanol_{theor}} \times 100$$

Where, $ethanol_{meas}$= *total ethanol produced by the fermentation (including ethanol from sampling) after 14 days of fermentation, in* $g.L^{-1}$ *initial volume.*

$ethanol_{theor}$= *theoretical ethanol production if consumed sugar (as measured) was fully metabolised into ethanol after 14 days of fermentation, in* $g.L^{-1}$ *initial volume.*

Percentage fermentation efficiency in the control fermentations, calculated using glucose, fructose, sucrose and maltose as the fermentable sugar only varied between 93% and 102% (results not shown). These values were higher than expected, as industrial ethanol yields from glucose fermentation by yeast are generally in the region of 80-90% of the theoretical value. Sugars, which have not been converted to ethanol, are utilised for yeast growth and formation of by-products (Jones *et al.* [1981]). Glucose, fructose, sucrose and maltose were the only fermentable sugar quantified from the fermentation medium (results shown in section 5.1.2.2). However, maltotriose is another fermentable sugar present in beer wort, and is readily metabolised by the NCYC 1236 yeast, as specified by the yeast strain supplier

(Appendix A). Maltotriose concentration in the beer wort was estimated from an average concentration in typical malt (Hough *et al.* [1982]) and from its content in the total sugar added (data provided by the manufacturer of the Brewing Liquid sugar). In addition, it was estimated that the percentage uptake of maltotriose was equivalent to the one of maltose, which has been measured. This estimation is therefore not accurate as the rate of maltotriose uptake is slower than the one of maltose. However, the results shown in Table 5-2 using both estimations, agreed with data from industrial fermentations, as described previously. Fermentation efficiency, using estimated maltotriose initial concentration and uptake, ranged from 78 to 90 % in the control fermentations. Therefore, the general behaviour of the fermentations carried out and the analytical methods used for ethanol and sugar quantification were reliable. The same fermentation efficiency (85%) was found for Standard 1100 fermentation, which has been carried out twice at different times. This confirmed a relatively good repeatability of the fermentation.

Table 5-2: Key ethanol and sugar concentrations with fermentation efficiencies in control fermentations (No stripping, NCYC 1236, 16°C)

Fermentation set	Initial sugar concentration (day 0)	Fermented sugars[1] (day 14)	Estimated maltotriose uptake (day 14)	Maximum theoretical ethanol[2] (day 14)	Ethanol production[3] (day 14)	Final ethanol concentration[4] (day 16)	Fermentation efficiency[5]
	$g.L^{-1}$ initial volume					$g.L^{-1}$ final volume	% w/w
Standard 1080	142	121	14	69	63	67	93
Standard 1100 (1)	175	135	12	78	66	68	85
Standard 1100 (2)	175	134	12	75	63	67	85
Non-agitated 1100	175	105	7	57	52	54	90
Non-aerated 1100	175	107	8	59	57	67	94

[1]Fermented sugars, as measured in the fermentation medium (does not include estimated maltotriose).
[2]Maximum theoretical ethanol, as calculated from fermented sugars (incl. estimated maltotriose), assuming that all the sugar was converted into ethanol.
[3]Ethanol production, including ethanol removed by sampling.
[4]Direct measurement of the amount of ethanol in the fermentation medium (no correction for volume loss through sampling).
[5]Fermentation efficiency is the ratio between ethanol production on day 14 and maximum theoretical ethanol.

5.1.2.1 Changes in medium ethanol concentration

Ethanol concentrations, measured by gas chromatography (Chapter 3), were expressed as grams per litre ($g.L^{-1}$) which were converted when necessary into %v/v, the unit most commonly used by brewers to express the alcoholic content of beer. Figure 5–1 shows the changes in ethanol concentrations during the various control fermentations. All fermentations showed a similar behaviour and an expected pattern in terms of changes in ethanol concentration during the course of fermentation. As found for typical yeast fermentations, the ethanol production curve for the control fermentations could be divided into four phases:

- Phase1 (day 0 to day 1) where the ethanol production rate was very slow (yeast growth is in lag phase).

- Phase 2 (day 1 to day 4), where the ethanol production rate was high (corresponds to exponential yeast growth).

- Phase 3 (day 4 to day max), where ethanol production still increased but at a much lower rate than in phase 2. Day max was the day at which the ethanol concentration reached a maximum. Day max varies with the fermentation conditions.

- Phase 4 (day max to day 16), where ethanol production rate was almost nil and ethanol concentration reached a plateau.

Standard conditions of fermentation involved continuous mechanical agitation of the fermentation medium and initial aeration of the beer wort. The maximum ethanol produced by the control fermentations in Standard 1080 and Standard 1100 on day 16 was the same for both fermentations at 67-68 $g.L^{-1}$ of the final volume (8.5-8.7 % v/v). This concentration corresponds to the ethanol tolerance limit of the yeast species under the conditions of fermentation. The theoretical ethanol tolerance, given by the supplier of yeast species used in this work, was 8% v/v (Appendix A). The difference between the manufacturer value and the value found in the present work was attributable to the difference in the environmental parameters and in the methods of evaluating ethanol tolerance (Casey and Ingledew [1985], [1986]).

In Standard 1080 and Standard 1100 fermentations, ethanol reached a plateau after approximately 10 days of fermentation. The rate of fermentation was slower for the lower gravity fermentation (1080) at the beginning of the fermentation, but gradually increased to reach the maximum ethanol concentration by day 14.

When the fermentation was not aerated (Non-aerated 1100), the initial rate of fermentation was the same as for Standard 1100 fermentation, but decreased after 5 days of fermentation. The time taken for ethanol concentration to reach the same ethanol concentration than in the standard fermentations was longer. It is only after 16 days of fermentation that ethanol concentration reached 67 $g.L^{-1}$ of final volume (similar to values for the standard fermentations with OG 1080 and OG 1100).

When the fermentation was not agitated (Non-agitated 1100), the initial rate of fermentation was the same as for the standard fermentation but decreased after five days. Ethanol concentration in this fermentation reached a plateau of 54 $g.L^{-1}$ of final volume only.

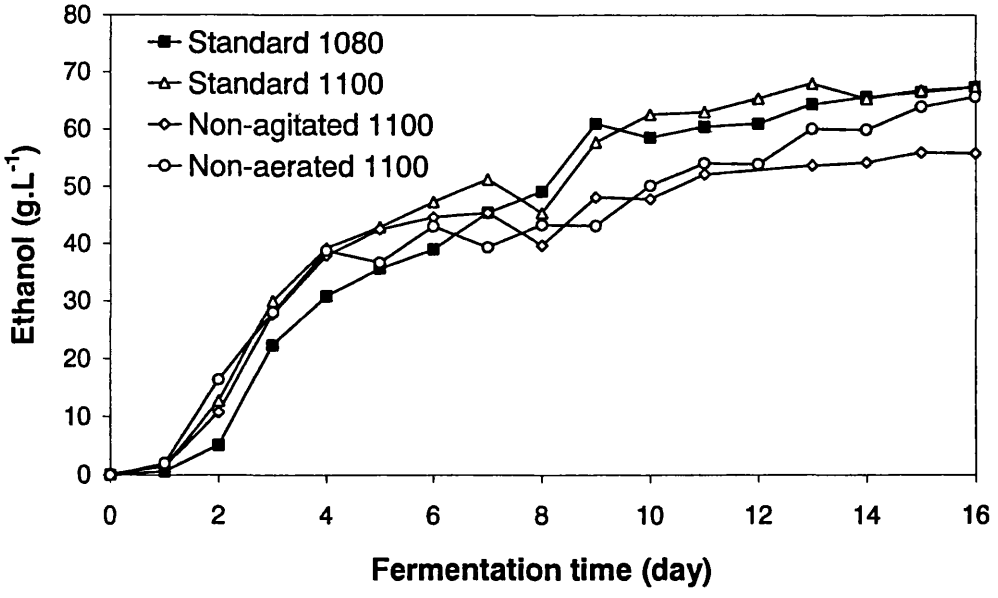

Figure 5–1: Changes over time in ethanol concentration in control fermentations (No stripping, NCYC 1236, 16°C).

5.1.2.2 Changes in sugar consumption

Sugar concentrations were measured on day 0 and day 14 of the fermentation. Maltose, glucose, fructose and sucrose were quantitatively analysed using an enzymatic technique, as described in Chapter 3. Due to the order of magnitude difference of concentration between some sugars, accuracy and precision of the determination of the minor sugars by the enzymatic method was impaired. Thus, differences between low sugar concentrations were probably insignificant. The coefficient of variation for the sugar concentrations varied between 1.5 and 5%.

Initial total sugar concentration (maltose, glucose, sucrose and fructose) was 142 $g.L^{-1}$ and 175 $g.L^{-1}$ in the fermentations with OG 1080 and OG 1100 respectively. The maltose/glucose/fructose/sucrose ratio was kept approximately constant in all the fermentations at approximately to 65:30.5:3:1.5.

The weight percent of sugar uptake was calculated as follows:

$$\%\ sugar\ uptake = \frac{sugar_{consumed}}{sugar_{fermentable}} \times 100$$

where, $sugar_{consumed}$ = *mass (g) of consumed/fermented sugars on day 14, estimated as follows:* $sugar_{consumed} = sugar_{day\,0} - sugar_{samples} - sugar_{day\,14}$

$sugar_{fermentable}$ = *mass (g) of fermentable sugars present in the wort at the beginning of the fermentation (maltotriose not included), estimated as follows:* $sugar_{fermentable} = sugar_{day\,0} - sugar_{samples}$

$sugar_{day0}$ = *initial mass (g) of sugar in the fermentation medium*

$sugar_{samples}$ = *total mass (g) of sugar removed through sampling*

$sugar_{day14}$ = *residual mass of sugar (g) in the medium after 14 days*

The mass of sugars lost through daily sampling was calculated using a predicted sugar consumption curve, and using the known volume of samples withdrawn from the fermentation medium. The predicted sugar consumption curve was estimated from measured sugar available on day 0 and day 14, assuming that the sugar curve followed the reverse of the ethanol production curve (Appendix E). It was assumed that all the different sugars followed the same consumption pattern. However, this assumption did not take into consideration that, for example, for most yeast

fermentations, maltose uptake starts when 60% of the wort glucose has been taken up by the yeast (Stewart *et al.* [1988] and Crumplen *et al.* [1989]. However, in the present study, this prediction provided an acceptable measurement of sugar mass lost through sampling.

As shown in Table 5-2, a total concentration of 135 $g.L^{-1}$ initial volume of sugars was consumed in Standard 1100 fermentation, which represented 77% of the fermentable sugars. In the non-agitated and non-aerated fermentations with OG 1100, between 105 and 107 $g.L^{-1}$ of sugars was consumed, which represented 60-61% of the fermentable sugars. In the lower gravity fermentation (Standard 1080), where only 142 $g.L^{-1}$ of fermentable sugars were present (compared to 175 $g.L^{-1}$ in the higher gravity fermentations), a higher sugar uptake was found. 121 $g.L^{-1}$ initial volume of sugars were consumed, which represented 85% of fermentable sugars.

Table 5-3, Table 5-4, Table 5-5 and Table 5-6 present a detailed examination of maltose, glucose, sucrose and fructose concentrations respectively, as estimated from day 0 and day 14 measurements and from the predicted sugar curve. In all the different fermentations, between 90 and 96 %w/w of either glucose, fructose or sucrose was consumed, so that only residual concentrations of these sugars remained in the fermentation medium after 14 days of fermentation. Maltose (Table 5-3), however, remained in the fermentation medium at a much higher level, and its residual concentration varied from one fermentation to another. In the Standard 1080 fermentation, 81% w/w of maltose was consumed, which left approximately 12 $g.L^{-1}$ final volume of maltose in the medium. In Standard 1100 fermentation, only 67 % w/w of maltose was consumed, which led to a remaining concentration of 34 $g.L^{-1}$ final volume. In the non-agitated and non-aerated fermentations, a lower uptake of maltose of 41 and 43 %w/w respectively led a remaining concentration of 65 and 62 $g.L^{-1}$ final volume of maltose.

Table 5-3: Maltose concentrations in control fermentations (without stripping).

	Initial conc.	Sampling	Fermented sugars	Residual sugars	Uptake	Residual sugars
	($g.L^{-1}$ initial volume)				% w/w	($g.L^{-1}$ final volume)
Standard 1080	94	7	76	11	81	12
Standard 1100 (1)	113	6	76	30	67	34
Standard 1100 (2)	113	9	75	29	66	34
Non-agitated 1100	113	11	46	56	41	65
Non-aerated 1100	113	12	49	52	43	62

Table 5-4: Glucose concentrations in control fermentations (without stripping).

	Initial conc.	Sampling	Fermented sugars	Residual sugars	Uptake	Residual sugars
	($g.L^{-1}$ initial volume)				% w/w	($g.L^{-1}$ final volume)
Standard 1080	42	3.3	39	0.3	93	0.3
Standard 1100 (1)	55	2.1	52	0.4	95	0.5
Standard 1100 (2)	55	2.9	52	0.2	95	0.2
Non-agitated 1100	55	2.9	52	0.2	95	0.3
Non-aerated 1100	55	2.8	51	1.1	93	1.3

Table 5-5: Fructose concentrations in control fermentations (without stripping).

	Initial conc.	Sampling	Fermented sugars	Residual sugars	Uptake	Residual sugars
	($g.L^{-1}$ initial volume)				% w/w	($g.L^{-1}$ final volume)
Standard 1080	3.7	0.3	3.4	0.0	92	0.0
Standard 1100 (1)	4.7	0.2	4.5	0.0	96	0.1
Standard 1100 (2)	4.7	0.2	4.5	0.1	96	0.1
Non-agitated 1100	4.7	0.2	4.5	0.0	96	0.1
Non-aerated 1100	4.7	0.2	4.4	0.1	94	0.1

Table 5-6: Sucrose concentrations in control fermentations (without stripping).

	Initial conc.	Sampling	Fermented sugars	Residual sugars	Uptake	Residual sugars
	($g.L^{-1}$ initial volume)				% w/w	($g.L^{-1}$ final volume)
Standard 1080	2.1	0.2	1.9	0.0	90	0.0
Standard 1100 (1)	2.4	0.1	2.3	0.0	96	0.0
Standard 1100 (2)	2.4	0.1	2.3	0.0	96	0.0
Non-agitated 1100	2.4	0.1	2.3	0.0	96	0.0
Non-aerated 1100	2.4	0.1	2.3	0.0	96	0.0

5.1.2.3 Changes in the rate of fermentation

The rate of fermentation was indirectly followed by the change in specific gravity (SG) of the beer medium during the fermentation. The specific gravity measures the density of the fermentation medium, which is the ratio between the mass of the fermentation medium and the mass of an equivalent volume of water. The faster the drop in SG, the faster the rate of fermentation.

Figure 5–2 presents the changes over time in measured specific gravity for all control fermentations. The rate of fermentation measured by the change in specific gravity agrees with the ethanol production rate shown in Figure 5–1. All fermentations followed a similar pattern. The rate of fermentation was fast in the first 4 days of fermentation, and then decreased until day 10-12, when it became almost nil. The fermentation rate at the beginning of the fermentation was slower for the lower gravity fermentation (OG 1080) than for higher gravity fermentations (OG 1100). This agrees with the lower ethanol production rate in the lower gravity fermentation at the beginning of the fermentation (Figure 5–1).

Fermentation rates for both Non-agitated 1100 and Non-aerated 1100 fermentations were similar to the ones of Standard 1100 fermentation until day 8. From this day, SG from both Non-aerated 1100 and Non-agitated 1100 fermentations remained higher than in Standard 1100 fermentation. In Non-agitated 1100 fermentation, the higher specific gravity after day 8 agrees with a lower ethanol production (Figure 5–1). Although the two specific gravity curves for both Standard 1100 fermentations (1) and (2) were not exactly the same, their profile was very similar, testament of a good repeatability of the fermentation. The differences were attributed to experimental error and to the intrinsic complexity of fermentation processes.

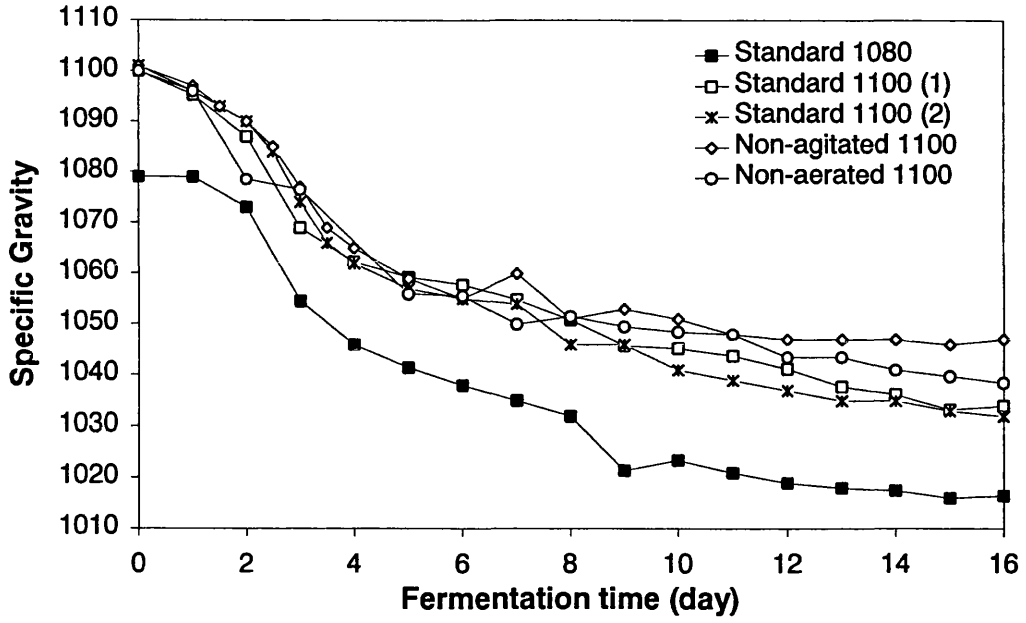

Figure 5–2: Changes over time in specific gravity during control fermentations (No stripping, NCYC 1236, 16°C).

5.1.2.4 Changes in medium pH

During an alcoholic fermentation, fall in pH is mainly due to the production of organic acids during yeast metabolism. The non-volatile acids comprise mainly of lactic, malic, citric and succinic acids, whereas the volatile fraction comprises of acetic acid and higher fatty acids. A typical decrease from pH 5.1 to pH 4.4 was found during the fermentation of control fermentations studied in the present work, which agrees with typical brewing fermentations (Hough *et al.* [1982]).

In the first two days of fermentation, pH remained constant at approximately 5-5.1. Medium pH then dropped sharply to 4.2-4.3 between day 3 and day 4. From day 4, it remained relatively constant until the end of the fermentation. Only a slight increase of 0.1-0.2 pH units occurred after 8 days of fermentation.

The variation in the non-aerated 1100 fermentation (slower decrease in pH between day 2 and day 7) could be attributed to the lack of oxygenation resulting in a decrease in the production of organic acids. However, many results from this fermentation set showed variations, which were more realistically attributed to experimental error and inaccurate measurements.

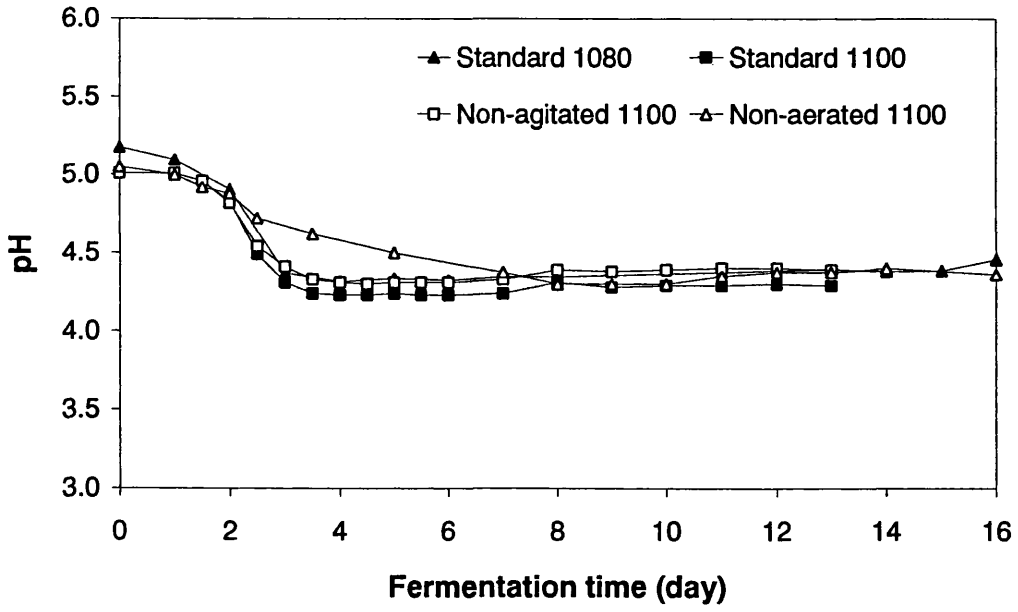

Figure 5–3: Changes over time in medium pH during control fermentations (No stripping, NCYC 1236, 16°C).

5.1.2.5 Main beer volatile production

Because of the limitations of the chromatographic technique used for the quantitative analysis of beer flavour compounds (Chapter 3), only isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde concentrations were measured in the beer medium. These five quantified compounds belong to the principal volatile constituents of beer along with *active*-amyl alcohol, β-phenyl ethanol and isoamyl acetate, which could not be analysed.

Table 5-7 compares the final concentrations of the measured volatile compounds in control fermentations carried out in the present work with concentrations of commercial beers, reported by Hough *et al.* [1982]. Levels of propanol, isobutanol, isoamyl alcohol, ethyl acetate and acetaldehyde were within the concentration range quoted for beers with ethanol levels between 4 and 9 % (v/v). Isoamyl alcohol was the most predominant volatile compound after ethanol, followed by isobutanol, propanol, ethyl acetate and acetaldehyde.

Table 5-7: Comparison between final concentrations of selected volatile compounds from the various fermentation sets and their respective concentrations in commercial beers.

	Fermentation sets				Commercial beers (Hough *et al.* [1982])	
	Standard 1080[1]	**Intermittent stripping 1080[2]**	**Standard 1100 (1) [1]**	**Non-aerated 1100[1]**	**Stout (9% v/v)**	**Pale Ale (4% v/v)**
Ethanol ($g.L^{-1}$)	67	55	68	66	70	31
Isoamyl alcohol ($mg.L^{-1}$)	123	223	350	193	169	61
Isobutanol ($mg.L^{-1}$)	61	50	54	75	98	33
Propanol ($mg.L^{-1}$)	55	17	43	51	60	48
Ethyl acetate ($mg.L^{-1}$)	42	N/A	46	26	69	23
Acetaldehyde ($mg.L^{-1}$)	N/A	N./A	20	23	0.5-10 (ppm)	4-34 (ppm)

[1]carried out with NCYC 1236 at 16°C, [2]carried out with a Wine yeast at 22°C.

5.1.3 General examination of stripped fermentations

The following sections compare the control fermentations, as examined in the previous section, with their appropriate stripped fermentations. Figure 5–4 shows a general pattern of ethanol changes for both control and stripped fermentations. As examined in section 5.1.2, the ethanol production curve can be divided into four phases. Phase one (day 0 –1) and phase two (day 1-3) were the same for both control and stripped fermentations as stripping was only activated when day 3 was completed. Stripped ethanol, although increasing during the course of phase 3, was always lower than control ethanol. At day max, where control ethanol had reached a plateau, stripped ethanol had reached its maximum value. After day max, stripped ethanol started to decrease.

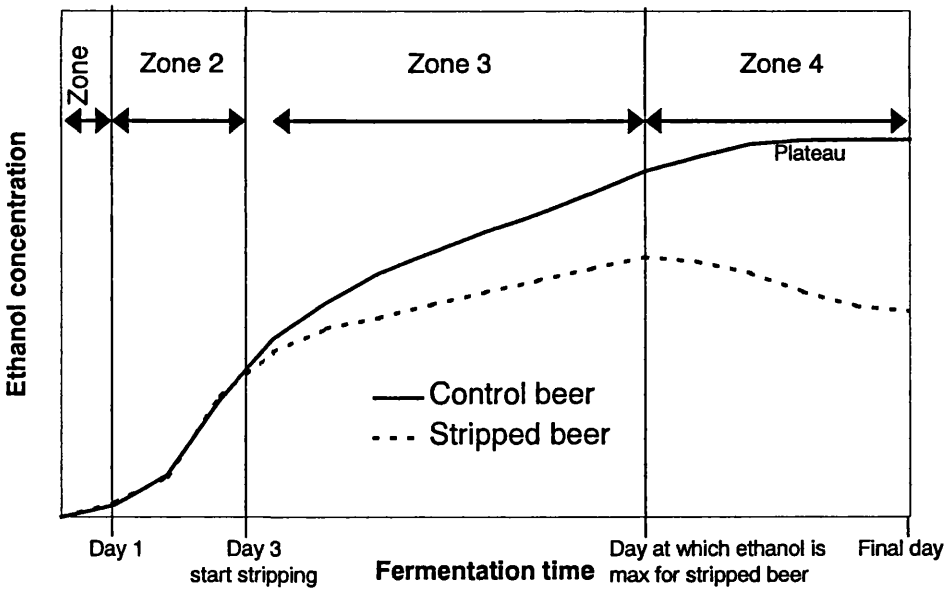

Figure 5–4: Schematic figure of ethanol changes in control and stripped fermentations.

Table 5-8 summarises stripped fermentations results in terms of initial sugar, fermented sugar, ethanol production (in $g.L^{-1}$ initial volume) on day 14, measured ethanol (in $g.L^{-1}$ final volume) on day 16, and fermentation efficiency. To assess the effect of stripping on the fermentation behaviour, Table 5-8 can be compared with Table 5-2, which presents equivalent information for control fermentations. Despite a higher sugar uptake, stripping resulted in a lower ethanol production (day 14). Therefore percentage fermentation efficiencies (calculated following the relation used in section 5.1.2 for the control fermentations, including condensate ethanol in ethanol production on day 14) were lower in the stripped fermentations than in the control fermentations. In the stripped fermentations percentage efficiencies ranged between 55 and 65% compared to 85%-94% in the control fermentations. As a first approximation, the results suggested that 32 to 45% of fermented sugar in the stripped fermentations has been metabolised into other products than ethanol. However, as described in Chapter 4, the inefficiency of the condensation system accounts for the low measured ethanol production. The following sections will cover this discrepancy in more detail.

Table 5-8: Key ethanol and sugar concentrations and fermentation efficiency for the stripped fermentations, carried out with NCYC 1236 at 16°C..

Fermentation set	Initial sugar concentration (day 0)	Fermented sugars [1] (day 14)	Estimated maltotriose uptake	Maximum theoretical ethanol[2] (day 14)	Ethanol production (day 14)[3]	Final medium ethanol concentration (day 16)[4]	Fermentation efficiency[5]
	$g.L^{-1}$ initial volume					$g.L^{-1}$ final volume	% w/w
Standard 1080	142	127	14	65	40	35	55
Standard 1100 (1)	175	147	14	78	55	40	65
Non-agitated 1100	175	130	11	67	47	33	65
Non-aerated 1100	175	151	15	77	48	39	57

[1]Fermented sugars, as measured in the fermentation medium (does not include estimated maltotriose).
[2]Maximum theoretical ethanol, as calculated from fermented sugars (incl. estimated maltotriose), assuming that all the consumed sugar was converted into ethanol.
[3]Ethanol production, as calculated including ethanol removed by sampling and recovered in the condensate (and trap when used).
[4]Direct measurement of the amount of ethanol in the fermentation medium (no correction for volume loss through sampling).
[5]Fermentation efficiency is the ratio between ethanol production on day 14 and maximum theoretical ethanol.

5.1.4 Effect of stripping on ethanol production

5.1.4.1 Ethanol in beer medium

When CO_2 stripping was activated after day 3, the ethanol level in the stripped media was continually maintained under that of the control ethanol concentration for all the fermentations. Stripped ethanol concentration in most of the fermentation sets reached a maximum level before decreasing to a final value. Table 5-9 summarises the results by comparing control and stripped fermentations. Ethanol concentrations in control and stripped fermentations, before stripping was initiated (day 3), were in the same range, between 20 and 28 $g.L^{-1}$ actual volume.

Stripped media from Standard 1100 fermentation (Figure 5–7), and from Non-aerated 1100 (Figure 5–9) exhibited similar changes in ethanol concentration. After being reduced in the first few days of stripping, the ethanol level gradually increased with an apparent rate of production similar to that of the control media and reached a value of 51 $g.L^{-1}$ actual volume (6.5% v/v). After this ethanol peak, further stripping forced the ethanol level to decrease to a final value of 39-40 $g.L^{-1}$ final volume, suggesting that stripping rate was higher than production rate.

For Standard 1080 fermentation (Figure 5–5), a similar pattern was found to Standard 1100 and Non-aerated 1100, with an ethanol peak of approximately 45 $g.L^{-1}$ actual volume. By the end of the fermentation, stripped medium ethanol was reduced to 35 $g.L^{-1}$ final volume (4.5 %v/v) which approached half the control ethanol concentration (67 $g.L^{-1}$ final volume). If a volume of water equal to the volume loss due to stripping was added back to the fermenter, final stripped ethanol concentration would actually be 27 $g.L^{-1}$ (3.4 % v/v).

No ethanol peak was found in Non-agitated 1100 (Figure 5–8). From day 4-5 of this fermentation, ethanol concentration in stripped medium gradually decreased to a final value of 33 $g.L^{-1}$ (4.2 % v/v).

Intermittent stripping fermentation was carried out with an initial wort with OG 1080, a temperature of 22°C and a wine strain yeast. The NCYC beer strain was not available at the time of experiment. Fermentation of the beer medium using a wine

yeast was found to be very slow, temperature was therefore set to 22°C to increase fermentation rate. Fermentations were allowed to proceed for extended periods and with three distinct stripping periods (day 4-7, 9-10, and 14-17). Ethanol concentration in the periodically stripped medium (Figure 5–6) was kept below 38 $g.L^{-1}$ (4.8% v/v) compare to 55 $g.L^{-1}$ (6.9% v/v) in the control medium, and finally reduced to 31 $g.L^{-1}$ (3.9 % v/v) by the end of the fermentation. During the first two periods of stripping (day 4-7 and day 9-10) removal of ethanol by stripping enabled ethanol concentration to be maintained at stable values of 26 $g.L^{-1}$ and 31 $g.L^{-1}$, respectively. The following non-stripping periods (day 7-9, day 10-13) allowed ethanol to build up in the beer medium to a concentration of 29 $g.L^{-1}$ and 38 $g.L^{-1}$ respectively. Ethanol production rates (approximately 3 $g.L^{-1}$ of medium per day) during these periods of non-stripping were the same and almost identical to the ones from the control beer. Finally, during the last stripping period (day 14 to day 17) ethanol concentration in the stripped beer fell down to 31 $g.L^{-1}$, and ethanol production rate in the control medium was reduced to an average of 1.3 $g.L^{-1}.day^{-1}$. It should be noted that intermittent stripped fermentation using the wine strain can not be directly compared to the other fermentations which used a brewing yeast.

It can also be observed that Figure 5–7 and Figure 5–8 (corresponding to Standard 1100 and Non-agitated 1100 respectively) using the optimised analytical method, showed a reasonably consistent effect of stripping through the fermentation. Figure 5–9 used less accurate analysis and whilst showing similar trends for the stripping effect, the increase level of noise made the absolute results less accurate.

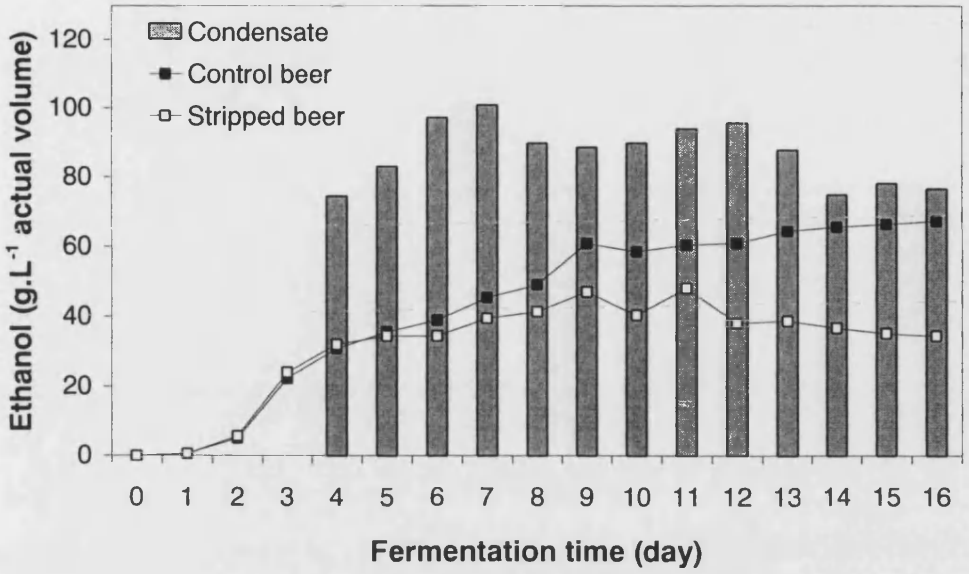

Figure 5–5: Changes over time in ethanol concentration in control medium, stripped medium and condensate during Standard 1080 fermentation (NCYC 1236, 16°C).

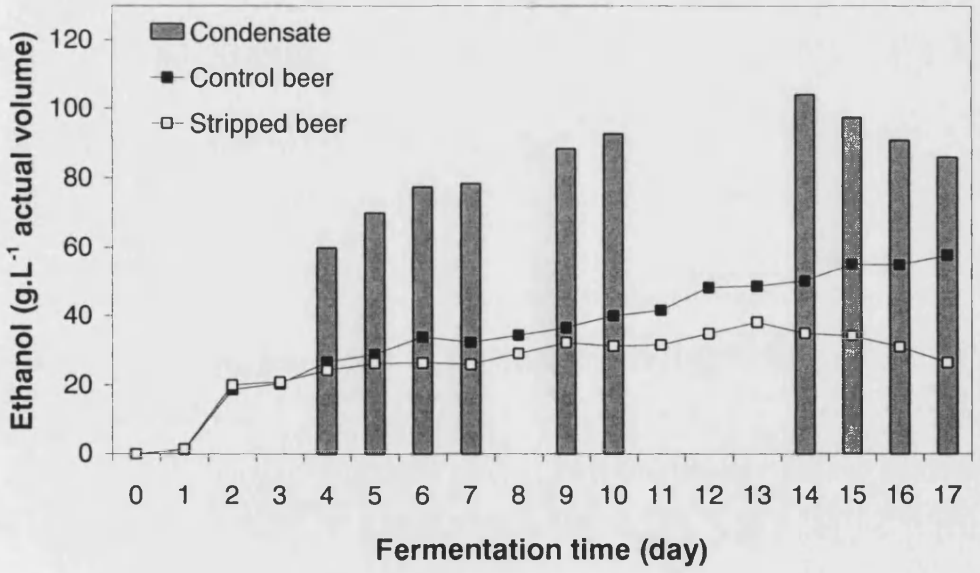

Figure 5–6: Changes over time in ethanol concentration in control medium, stripped medium, and condensate during Intermittent Stripping 1080 fermentation (Wine yeast, 22°C).

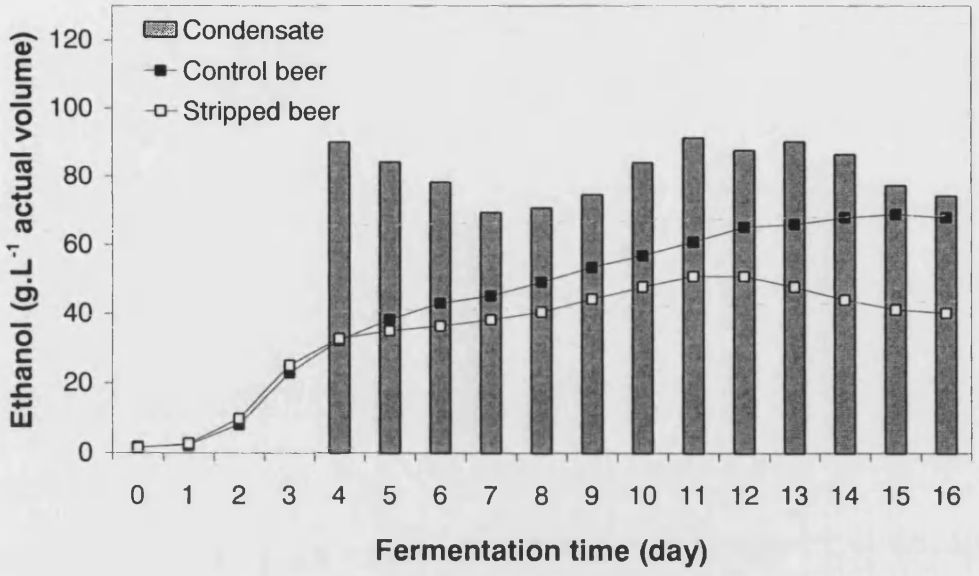

Figure 5–7: Changes over time in ethanol concentration in control medium, stripped medium and condensate during Standard 1100 fermentation (NCYC 1236, 16°C).

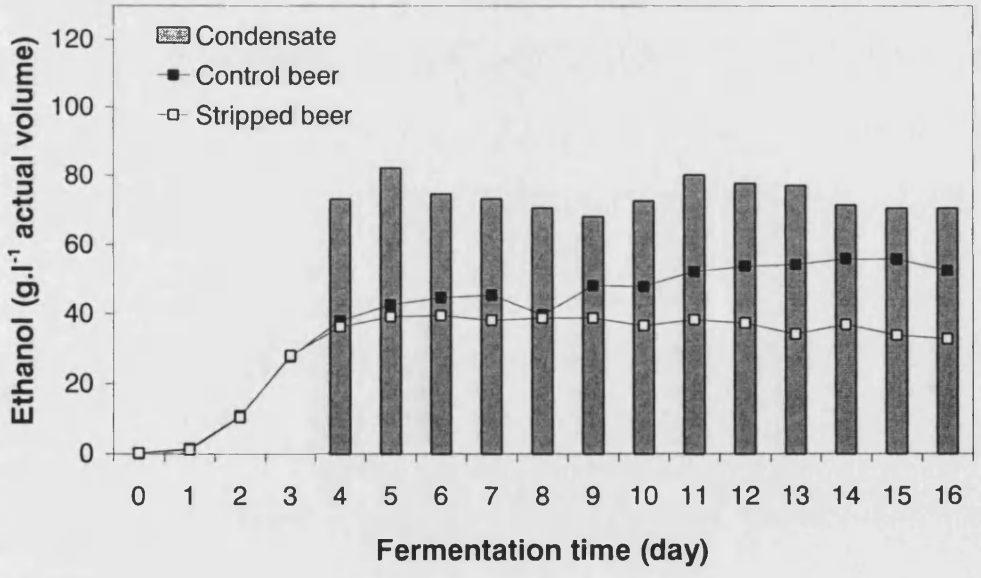

Figure 5–8: Changes over time in ethanol concentration in control medium, stripped medium and condensate during Non-agitated 1100 fermentation (NCYC 1236, 16°C).

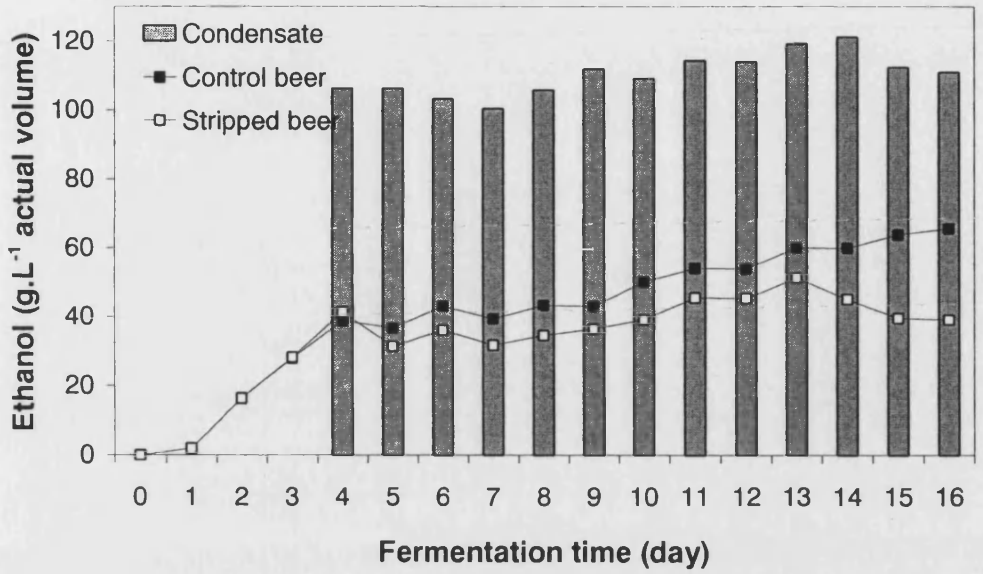

Figure 5–9: Changes over time in ethanol concentration in control medium, stripped medium, and condensate during Non-aerated 1100 fermentation (NCYC 1236, 16°C).

Table 5-9: Comparison between ethanol concentrations in control and stripped fermentations.

Fermentation set	Ethanol concentration ($g.L^{-1}$ actual volume) at day 3		Final ethanol concentration ($g.L^{-1}$ final volume) at day 16		Ethanol peak in stripped beer ($g.L^{-1}$ actual volume)	
	Control	Stripped	Control	Stripped	Day	Stripped
Standard 1080[1]	24	22	67	35	9-11	45
Periodical stripping 1080[2]	20	21	55	31	13	38
Standard 1100 (1) [1]	23	25	68	40	11	51
Standard 1100 (2) [1]- control only	28	N/A	67	N/A	N/A	N/A
Non-agitated 1100[1]	28	28	56	33	5	40
Non aerated 1100[1]	28	28	66	39	13	51

[1]carried out NCYC 1236 at 16°C, [2]carried out with a Wine yeast at 16°C.

5.1.4.2 Ethanol in condensate

Average condensate ethanol concentration and condensate volume are presented in Table 5-10. Ethanol concentration in condensate varied from 74 $g.L^{-1}$ (Non-agitated 1100) to 111 $g.L^{-1}$ (Non-aerated 1100), and condensate volume from 56 $ml.day^{-1}$ (Standard 1100) to 68 $ml.day^{-1}$ (Non-aerated 1100). From the calculated average condensate to medium ethanol ratio, it can be deducted that condensate ethanol was 2 to 3 times more concentrated than in the fermentation medium.

Table 5-10: Condensate ethanol and volume, and condensate/medium ethanol ratio.

Fermentation set	Average condensate ethanol ($g.L^{-1}$)	Average condensate volume ($ml.day^{-1}$)	Average condensate/ medium ethanol ratio	Ratio Standard deviation
Standard 1080[1]	87	66	2.3	0.3
Periodical stripping 1080[2]	85	81	2.9	0.2
Standard 1100[1]	82	56	2.0	0.3
Non-agitated 1100[1]	74	59	2.0	0.1
Non-aerated 1100[1]	111	68	2.8	0.3

[1]carried out with NCYC 1236 at 16°C, [2]carried out with a Wine yeast at 22°C.

The higher fermentation temperature used in the intermittent stripped experiment (22°C instead of 16°C) resulted in the highest ratio of condensate to medium ethanol (2.9) and the highest average condensate volume (81 $ml.day^{-1}$). A higher fermentation temperature increased the extraction rate of volatile compounds such as ethanol. The actual average ethanol concentration in the condensate was 85 $g.L^{-1}$ which was approximately the same as with the Standard 1080 fermentation. However, because of lower stripped medium ethanol in Intermittent Stripping 1080 (38 $g.L^{-1}$ maximum) than in Standard 1080 (45 $g.L^{-1}$ maximum), condensate to medium ratio became higher than in Standard 1080.

Non-aerated fermentations gave also rise to a higher condensate ethanol concentration of 2.8. As the different fermentation sets were carried out at different

period of the year, room temperature varied (between 15 and 25°C) from one fermentation to another, which resulted in changes in condensation temperature (by heat loss from the coil). Condensate ethanol for Non-aerated 1100 fermentation (111 $g.L^{-1}$) was higher than in the other fermentation sets (between 74 and 87 $g.L^{-1}$). This suggests that a lower condensate temperature would have been in use at the time of operation, which would have allow for more volatile compounds to be captured by the condensation unit.

Changes in condensate ethanol (Figure 5–5 to Figure 5–9) followed a similar trend for all the fermentations. Figure 5–7, which shows the changes in ethanol concentration for Standard 1100 fermentation, is the most explicit profile. From day 7 to day 11-12, condensate ethanol increased at a similar rate as stripped medium ethanol. This period was then followed by a decrease in ethanol in both condensate and medium. To a certain extent, this was also seen in the other investigated fermentations, despite experimental variation. Another interesting result in Figure 5–7, was the decrease in condensate ethanol from day 4 to day 7, while stripped medium ethanol increased. A similar trend was demonstrated in the other two fermentations with OG 1100 (Non-agitated and Non-aerated). Due to the high metabolic rate of yeast at the beginning of a fermentation, it was suggested that the yeast cells were surrounded by a high local concentration in ethanol. This high local ethanol concentration would represent a higher driving force for gas stripping, and result in a higher extraction rate. No experimental work has been carried out to confirm this assumption.

The relationship between condensate ethanol and medium ethanol is illustrated in Figure 5–10. Despite a variation between the linear fit for Standard 1080, Standard 1100 and Non-agitated 1100, the slopes were of the same order of magnitude. At the contrary, the slope for Intermittent stripping 1080, which operated at a higher fermentation temperature of 22°C, was larger, which confirmed the results found in Table 5-10. Although the results for Non-aerated 1100 fermentation were not as explicit as for the other fermentations, a higher condensation temperature (as mentioned in a previous paragraph) would explain the shift of the slope towards the one of Intermittent 1080.

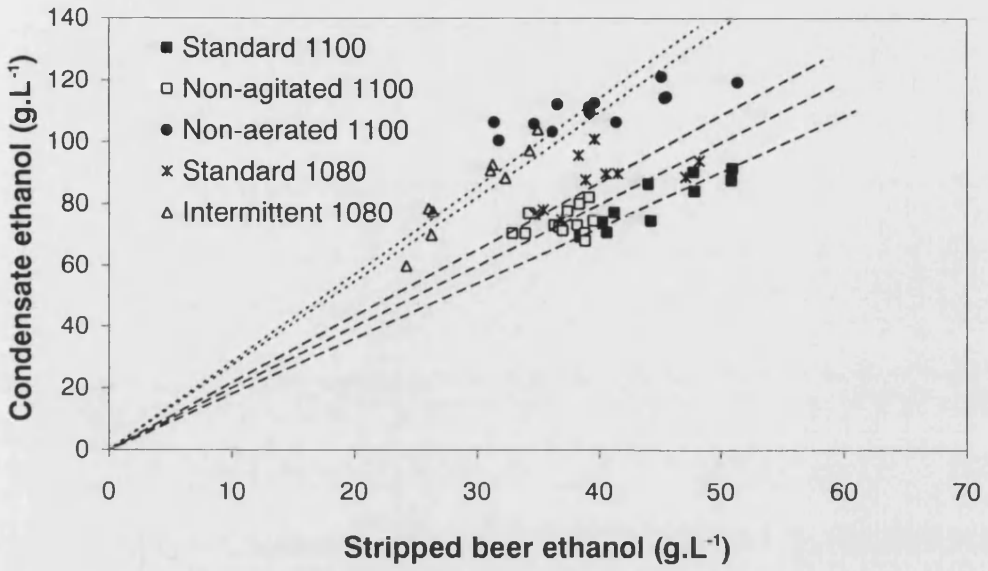

Figure 5–10: Relationship between stripped beer medium and condensate ethanol concentrations. All fermentations were carried out with NCYC 1236 at 16°C, except for Intermittent stripping 1080, which was carried out with a Wine yeast at 22°C.

5.1.4.3 Net ethanol production

Net ethanol production was calculated by adding the mass of medium ethanol at the end of the fermentation (day 14) with the total mass of ethanol removed by sampling and recovered in the condensate and trap (when used). For all the fermentations, net ethanol production in the stripped fermentations was always lower than in the control fermentations (Table 5-11). Even with the fermentations operating with the additional condensation step using a trap at -40°C (Standard and Non-agitated fermentations with OG 1100), net ethanol production was still lower than in the control. In Standard 1100 fermentation, control ethanol production was 66 $g.L^{-1}$ compared to only 55 $g.L^{-1}$ in the stripped fermentation. Results on synthetic stripping described in Chapter 4, showed that the condensation unit used during the fermentations was not efficient. Therefore, the data presented for the stripped fermentations, represents only apparent ethanol production, rather than true ethanol production.

Table 5-11: Net ethanol production in control and stripped fermentations at the end of the fermentation (day 14).

		Total ethanol produced on day 14 ($g.L^{-1}$ initial volume)				
		Medium	**Sampling**	**Condensate**	**Trap**	**Total**
Standard 1080	**Control**	59	4	N/A	N/A	63
	Stripped	31	3	7	N/A	40
Standard 1100 (1)	**Control**	62	4	N/A	N/A	66
Standard 1100 (2)	**Control**	57	5	N/A	N/A	62
Standard 1100 (1)	**Stripped**	34	3	5	12	55
Non-agitated 1100	**Control**	48	5	N/A	N/A	52
	Stripped	29	4	5	10	47
Non-aerated 1100	**Control**	52	5	N/A	N/A	57
	Stripped	35	4	8	N/A	48

5.1.5 Effect of stripping on sugar uptake

The wort sugars, maltose, glucose, fructose and sucrose, were quantified on day 0 and day 14 of the fermentation only. Table 5-12 presents percentage sugar uptake for both control and stripped fermentations. Sugar uptake in stripped fermentations was estimated from the relationship defined in section 5.1.2.2 for control fermentations. As described in that section, sugar loss from daily sampling, was assessed using a predictive sugar consumption curve, which relied upon both sugar measurements on day 0 and day 14.

For all the fermentation sets, stripping resulted in a higher total sugar uptake compared to control fermentations. Glucose, sucrose and fructose uptake was fairly identical in both control and stripped fermentations, ranging from 91 to 96% of the initial fermentable sugar. The higher sugar uptake in stripped fermentations was mainly due to the higher maltose uptake. The consumption of maltose in stripped fermentations ranged from 64 to 88% compared to 41% to 82% in control fermentations (Table 5-12). When aeration and agitation were omitted in control fermentations with OG 1100, only 60-61% of total sugar was consumed compared

77% in standard control fermentation. Omission of aeration in stripped fermentations did not change sugar uptake compared to standard stripped fermentations. As much as 86% of fermentable sugars were consumed in the non-aerated stripped fermentation, compared to 84% in standard stripped fermentation. Omission of agitation in the stripped fermentations, however, led to a decrease of the sugar uptake compared to standard 1100 fermentation. Only 75% of fermentable sugars were consumed compared to 84% in standard fermentations. Figure 5–11 presents a comparison between initial fermentable sugar with total fermented sugar in both control and stripped fermentations.

The differences in total sugar uptake between all the fermentations were mainly due to the differences in maltose uptake. Fermented maltose in stripped fermentations on day 14 was consistently higher than in control fermentations (Figure 5–12). In standard fermentation with OG 1080, 11% of the initial maltose remained in the control medium at day 14, whereas only 6% remained in the stripped beer. In the standard fermentation at a higher original gravity (OG 1100) which implies a higher initial maltose concentration, 27% of this initial maltose remained in the control medium, whereas only 18% remained in the stripped medium. As described for total sugars uptake, omission of aeration did not decrease the uptake of maltose in stripped fermentations, whereas omission of agitation led to a 18% decrease in maltose uptake compared to standard stripped fermentation.

Glucose uptake was almost identical in control and stripped media for the various fermentations. It varied from 92 to 95 % in the control fermentations and from 92 to 96% in the stripped fermentations. Comparison between initial fermentable glucose and fermented glucose in stripped and control fermentations are presented in Figure 5–13. As Standard 1080 fermentation had an initial fermentable of only 42 $g.L^{-1}$ compared to 55 $g.L^{-1}$ in the fermentations with a higher original gravity of 1100, only 39 $g.L^{-1}$ of glucose was fermented compared to 51-52 $g.L^{-1}$ in the OG 1100 fermentations. The 3-4 $g.L^{-1}$ difference between initial glucose concentration and fermented glucose concentration was mainly due to glucose removed by sampling. There was only between 0.2 and 1.1 $g.L^{-1}$ of glucose remaining in the medium in both control and stripped fermentations at the end of the fermentation (day 14). Accurate quantification of low glucose concentrations using the enzymatic technique could

have been impaired by the relatively large quantity of maltose remaining in the sample. Therefore the differences between 92 and 96% of glucose uptake in the control and stripped medium from the different set of fermentation were probably insignificant.

Results concerning fructose (Figure 5–14) and sucrose (Figure 5–15) were similar to the ones found for glucose. Only a very small proportion of both these sugars (less than 0.1 $g.L^{-1}$) remained at the end of the fermentation in control and stripped fermentations, as between 91 and 96% of fermentable fructose and sucrose were consumed by day 14. The accuracy and precision of the determination of fructose and sucrose could have been impaired by the relatively large amount of glucose and maltose (i.e. when the ratio of maltose or glucose to fructose or sucrose was higher than 10 to 1).

Measured produced ethanol (taking into account condensate ethanol in addition to medium and samples ethanol for the stripped fermentations, neglecting trap ethanol and losses due to partial condensation) on day 14 was plotted against total fermented sugar (Figure 5–16). For comparison between stripped and control fermentations, a linear fit was imposed onto the data. In control fermentations, the non-agitated and non-aerated fermentations resulted in lower ethanol production and sugar uptake compared to the standard fermentations. However, it was not expected that the relationship between produced ethanol and fermented sugar was strictly linear, as the difference in fermentation conditions could result in different ethanol yields. For example, a higher gravity fermentation could produce a higher proportion of glycerol due to higher osmotic pressure. This relationship between produced ethanol and consumed sugars was previously represented by the percentage efficiencies of the fermentations (83% for Standard 1080 and 78% for Standard 1100). If the control fermentations and stripped fermentations are compared in terms of gradient of the linear fit, the gradient for the stripped fermentations was lower than for the control fermentations. The higher the fermented sugar, the lower the apparent ethanol production in the stripped fermentations. It could also be interpreted as the higher the produced ethanol in the medium, the higher the driving force for stripping, the higher the extracted ethanol and consequently the higher the ethanol loss (due to the inefficiency of the condensation unit resulting in partial condensation).

Table 5-12: Comparison between % uptake of the total sugars and individual sugars in control and stripped fermentations.

Fermentation set	Total sugars		Maltose		Glucose		Sucrose		Fructose	
	Control	Stripped	Control	Stripped	Control	Stripped	Control	Stripped	Control	Stripped
Standard 1080	85	89	82	88	92	92	92	92	91	91
Standard 1100	77	84	68	78	95	96	96	96	95	95
Non-agitated 1100	60	75	41	64	94	94	96	96	95	95
Non-aerated 1100	61	86	44	83	93	93	96	96	94	94

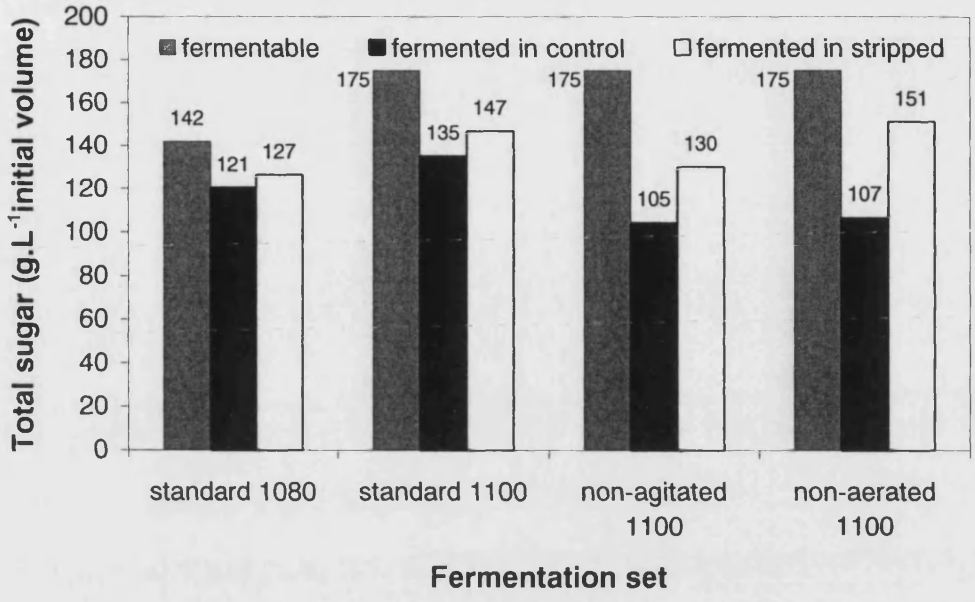

Figure 5–11: Fermented total sugars in control and stripped fermentations on day 14 compared to initial fermentable sugars on day 0.

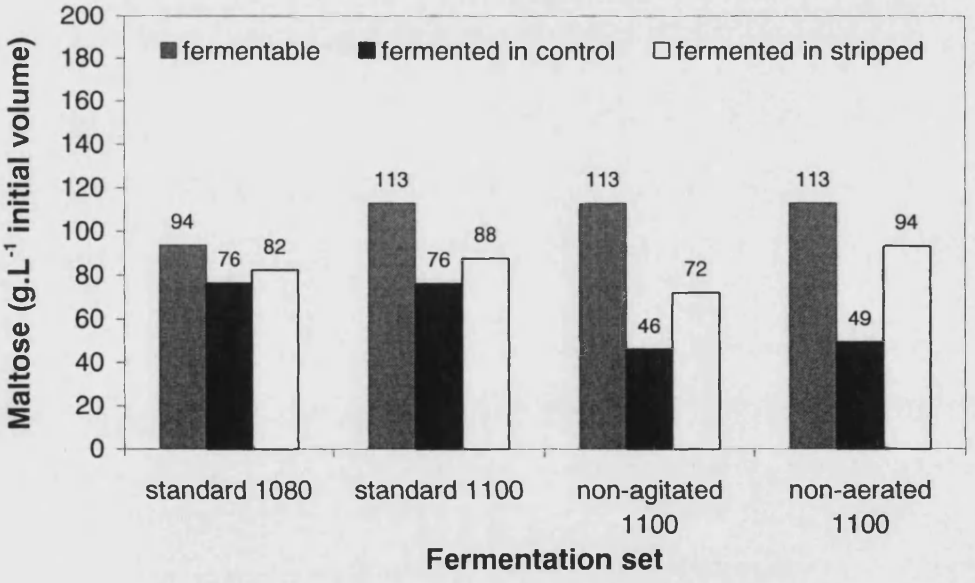

Figure 5–12: Fermented maltose in control and stripped fermentations on day 14 compared to initial fermentable maltose on day 0.

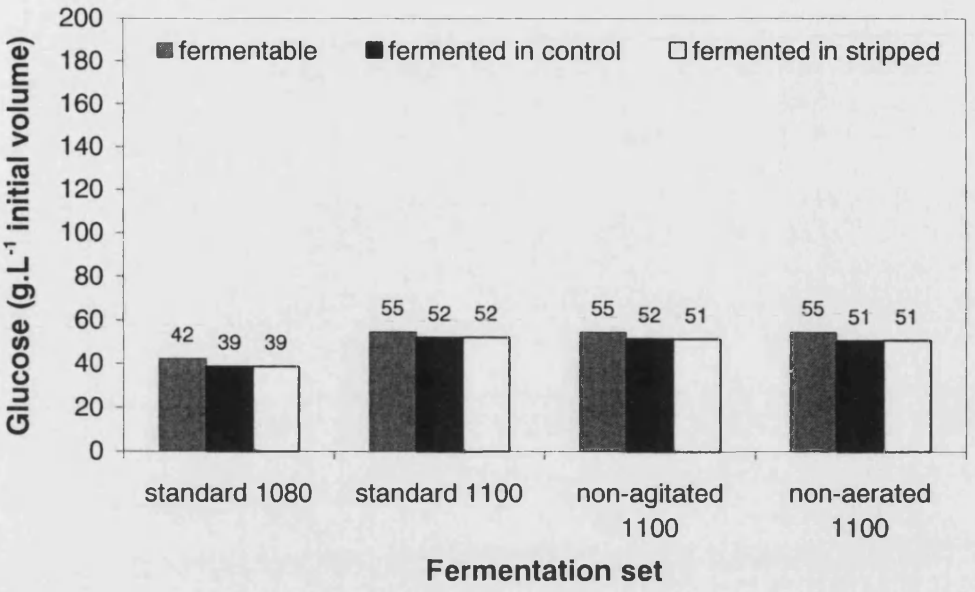

Figure 5–13: Fermented glucose in control and stripped fermentations on day 14 compared to initial fermentable glucose on day 0.

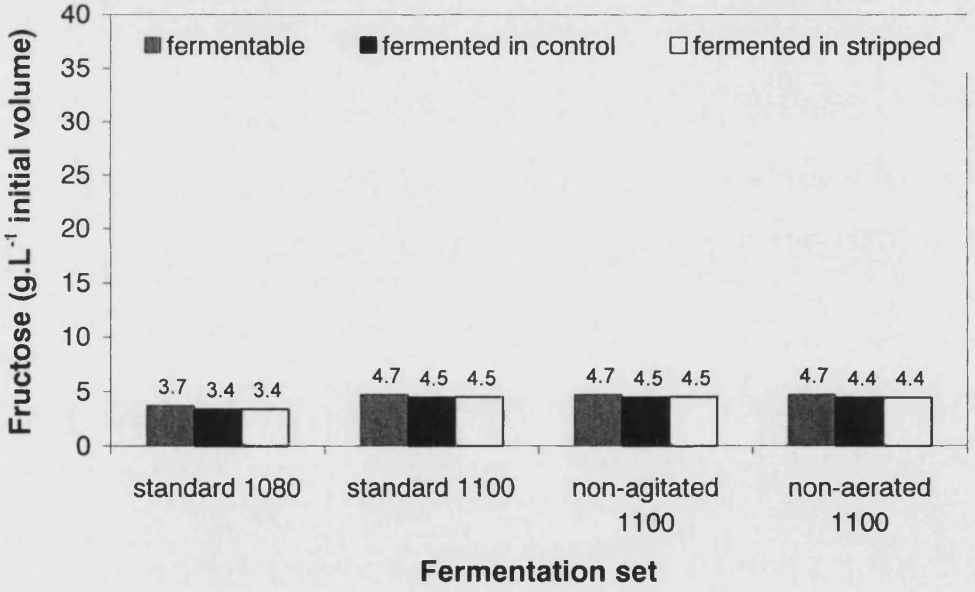

Figure 5–14: Fermented fructose in control and stripped fermentations on day 14 compared to initial fermentable fructose on day 0.

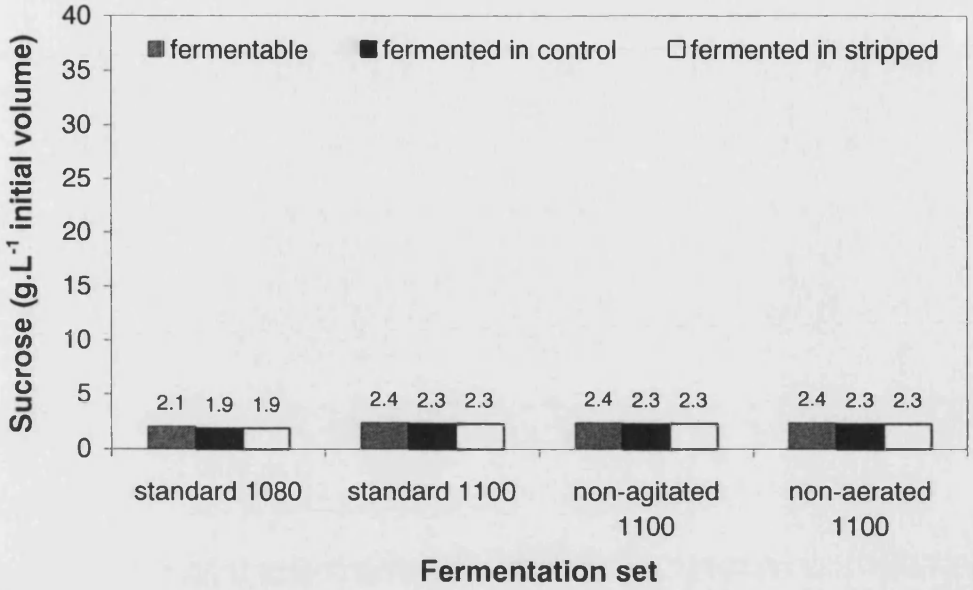

Figure 5–15: Fermented sucrose in control and stripped fermentations compared to initial fermentable sucrose on day 0.

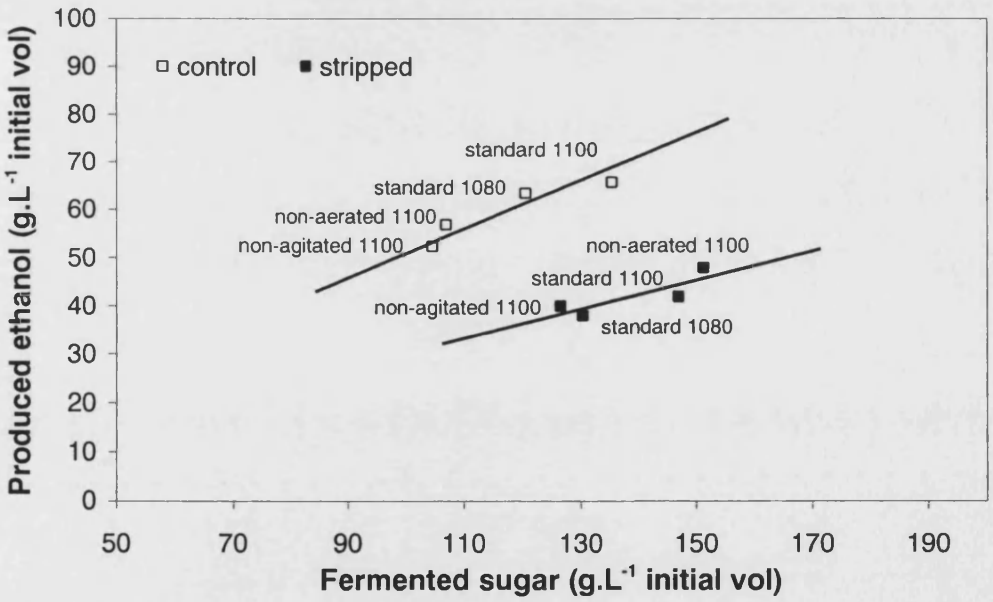

Figure 5–16: Relationship between measured produced ethanol and total fermented sugar for the different fermentations. For comparison purposes, trap ethanol (in opposition to condensate ethanol) was ignored in the calculation of ethanol production for the stripped fermentations, as only two of the stripped fermentations were operated with the additional trap (refer to page 107 for detailed information).

5.1.6 Effect of stripping on medium specific gravity

Measured specific gravity (SG) for both control and stripped fermentations are presented below along with corrected stripped SG. Indeed, to compare the rate of fall in SG between stripped and control medium, measured stripped SG was corrected for the volume loss due to removal of water and other volatile compounds by stripping. Table 5-13 presents key SG measurements in control and stripped fermentations for the different sets.

Corrected stripped SG ($SG_{corrected}$) was calculated as follows:

$$SG_{corrected} = \frac{m_{cond} + SG_{measured} \times Vol_{medium}}{(Vol_{medium} + Vol_{cond})}$$

Where: m_{cond} = *cumulative mass of condensate (and trap when used) in g*

$SG_{measured}$ = *measured specific gravity in* $g.ml^{-1}$

Vol_{medium} = *volume of actual fermentation medium in ml*

Vol_{cond} = *cumulative volume of condensate (and trap when used) in ml*

In most of the fermentations sets (Figure 5–17 to Figure 5–20), the same relation between ethanol concentration and SG in the stripped fermentations was found. While stripped ethanol concentration peaked up to a maximum value between day 10 and 12, measured stripped SG gradually decreased to a minimum value. After that point, further stripping led to a dramatic decrease in stripped ethanol concentration, and a slight increase or a stable value of stripped SG. In the non-agitated fermentation (Figure 5–21), where no ethanol peak was found during stripped ethanol, measured SG in the stripped fermentation was the same as in the control fermentation. This is probably coincidental, as the fermentation rate for the control medium of this fermentation was slower than for the standard fermentation.

When corrected for volume loss, stripped SG was in all the fermentation sets lower than the measured stripped SG. In Standard 1100 (Figure 5–19) and Non-agitated 1100 (Figure 5–21) fermentations, where an additional trap (-40°C) was used in addition to the coil condenser (0°C), corrected stripped SG was also lower than control SG after day 6. Correction for volume loss was calculated with the actual condensate and trap (when used) collected, and did not account for the loss of

volatiles not recovered by the condensation unit in use. In the other fermentation sets, only a condensate at 0°C was collected, therefore the corrected stripped SG was not as diminished as with Standard 1100 and Non-agitated 1100 fermentations.

The rate of fermentation of the control medium of Non-aerated 1100 fermentation was slower than in the control medium of Standard 1100 fermentation. Consequently, measured stripped SG (without correction) in Non-aerated 1100 fermentation after day 7, was lower than control SG (Figure 5–20). Contrary to all the other fermentations investigated, final measured stripped SG in this fermentation was lower than final control SG. Correction for volume loss led to a further decrease in stripped SG as found for the other fermentations.

The intermittent stripping fermentation with OG 1080 can not be directly compared with the Standard 1080 fermentation, due to the use of a different yeast strain. However, similar trends to Standard 1080 and Standard 1100 fermentations were found.

In summary, the results suggests that stripping led to a faster rate of fermentation, as measured by the lower specific gravity in the stripped fermentations compared to the control fermentations for fermentations carried out with the additional trap.

Table 5-13: Specific gravity measurements of control and stripped media on day 0 and day 14.

Fermentation set	Initial OG	Final SG (day 14)			Condensation Unit
		Control	Stripped	Corrected stripped	
Standard 1080[1]	1080	1018	1024	1020	0°C only
Intermittent stripping 1080[2]	1080	1035	1038	1033	0°C only
Standard 1100 (1) [1]	1100	1036	1042	1031	0°C and –40°C
Standard 1100 (2)-control only[1]	1100	1035	N/A	N/A	N/A
Non-agitated 1100[1]	1100	1047	1046	1034	0°C and –40°C
Non-aerated 1100[1]	1100	1041	1032	1025	0°C only

[1]carried out with NCYC 1236 at 16°C, [2]carried out with a Wine yeast at 22°C.

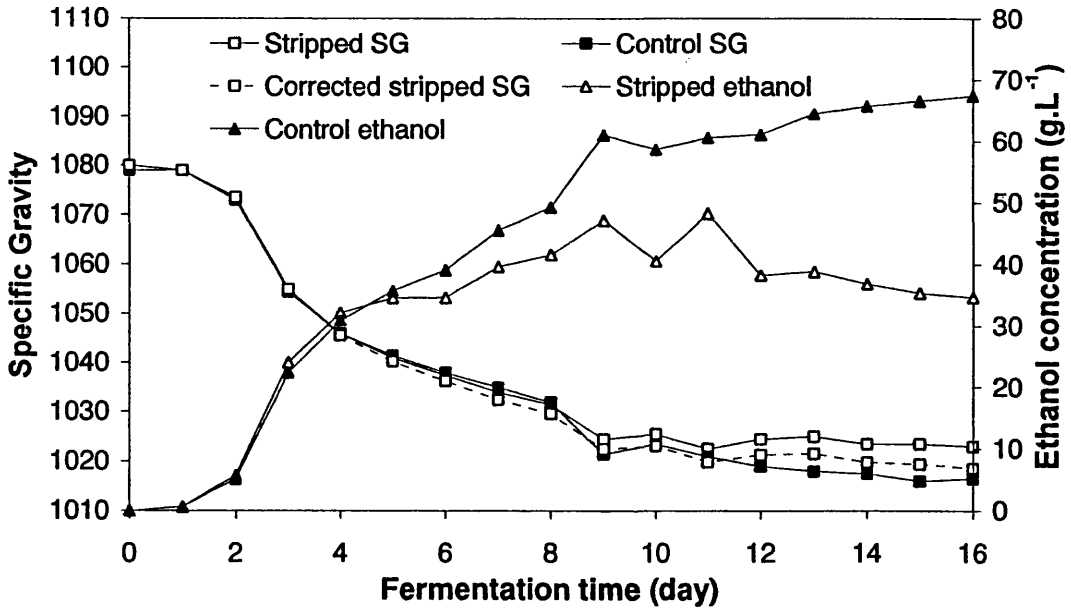

Figure 5–17: Changes in SG and ethanol concentration in control and stripped medium during Standard 1080 fermentation (NCYC 1236, 16°C).

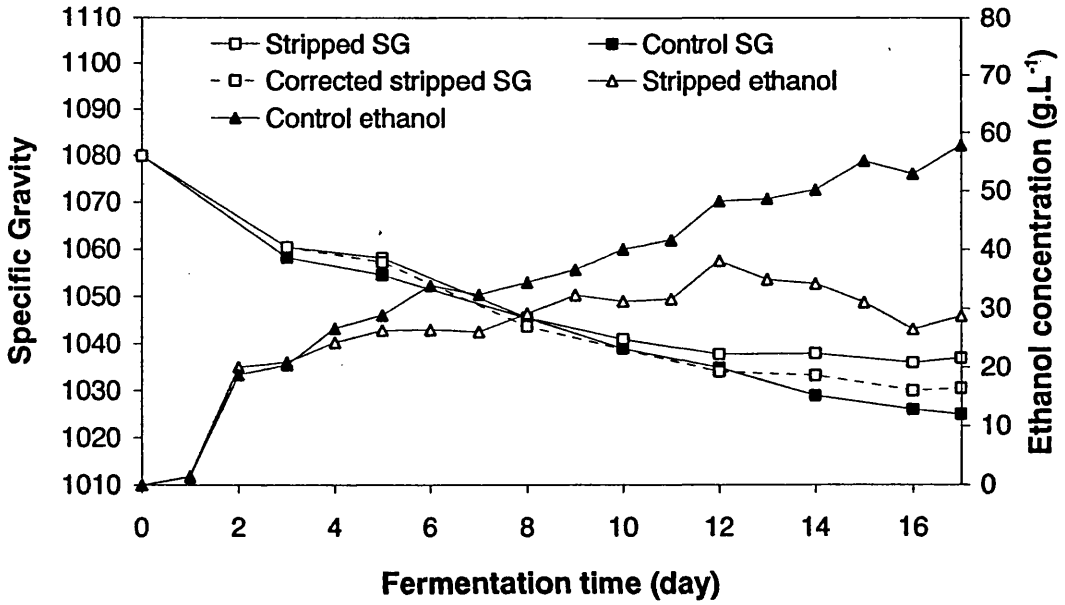

Figure 5–18: Changes in SG and ethanol concentration in control and stripped medium during Intermittent Stripping 1080 fermentation (Wine yeast, 22°C).

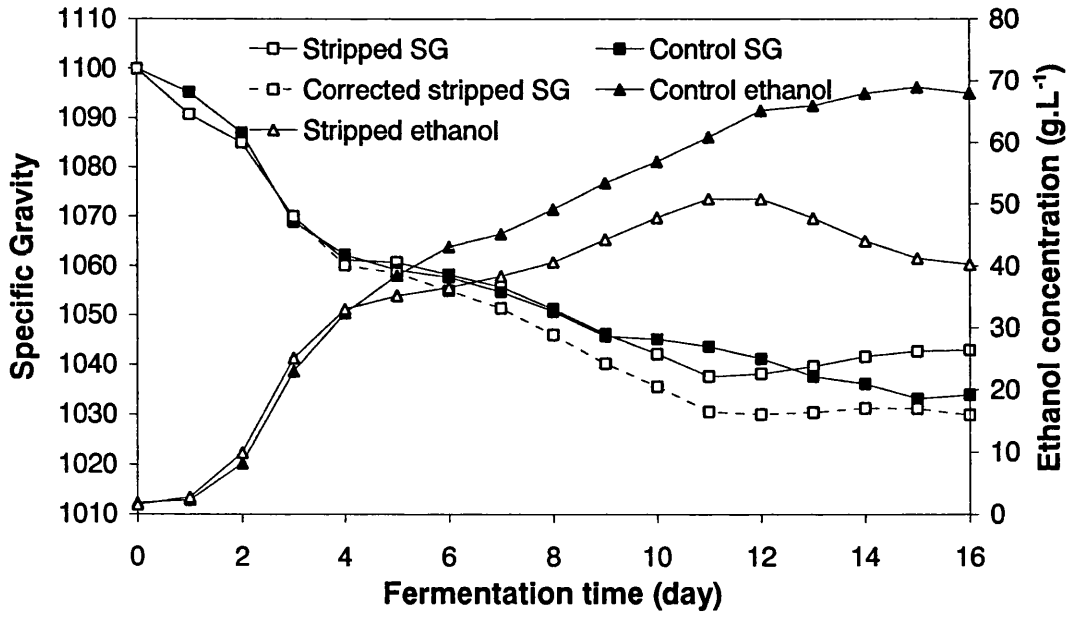

Figure 5–19: Changes in SG and ethanol concentration in control and stripped medium during Standard 1100 fermentation (NCYC 1236, 16°C).

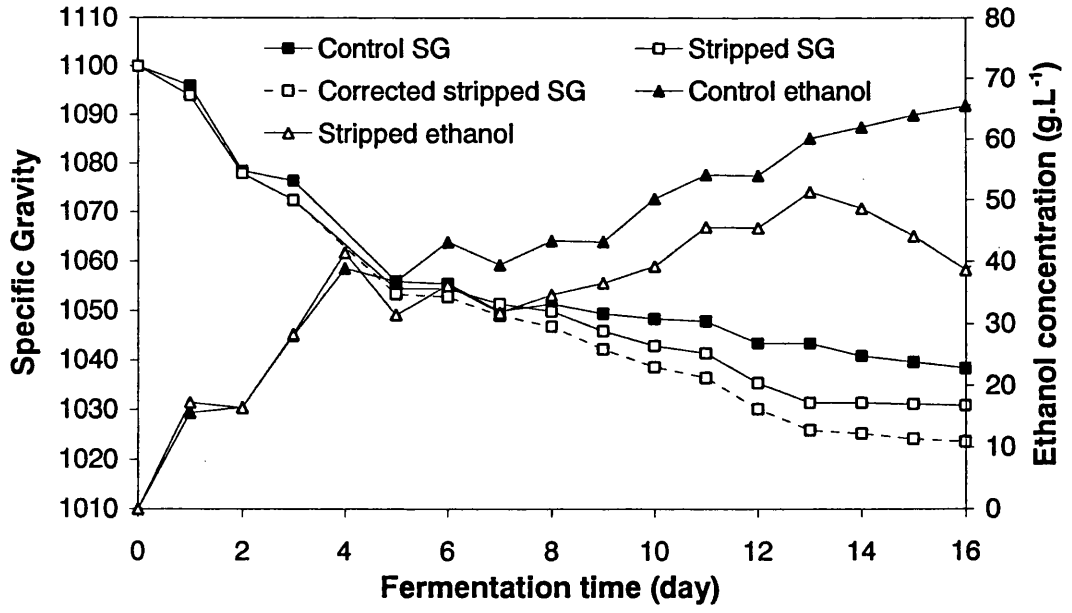

Figure 5–20: Changes in SG and ethanol concentration in control and stripped medium during Non-aerated 1100 fermentation (NCYC 1236, 16°C).

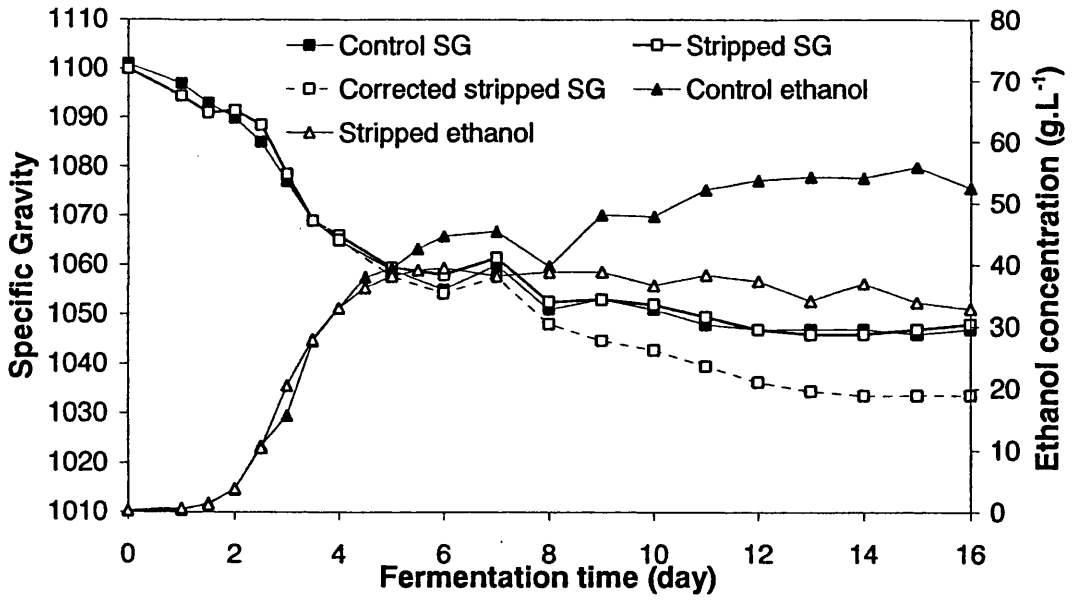

Figure 5–21: Changes in SG and ethanol concentration in control and stripped medium during Non-agitated 1100 fermentation (NCYC 1236, 16°C).

5.1.7 Effect of stripping on medium pH

Changes in pH were monitored in all the fermentations. Similar pH profiles were obtained under the different fermentation conditions, in control and stripped fermentations. Only changes in pH in standard fermentation with OG 1080 are shown (Figure 5–22), as the other fermentations performed in a similar manner. Table 5-14 summarises the results obtained for the different sets of fermentations by presenting initial and final pH measurements.

In all fermentations, initial pH was approximately equal to 5.10 (± 0.05). A rapid drop in pH occurred in the first three days of fermentation due to production of organic acids, and remained relatively stable. Only a slight increase of pH occurred in control fermentations from day 3 until the end of the fermentation. Final pH in control fermentations was equal to 4.30-4.40 (± 0.05). Stripping resulted in a small drop of pH from day 6-9 compared to the control fermentation. Final stripped pH was consistently lower than control pH by 0.1 or 0.2 units difference, and was equal to

4.20-4.30 (± 0.05) in the continuously stripped fermentations. Differences in pH between the different fermentations were minimal. When fermentation was not initially aerated, pH did not drop as sharply in the first few days as in the standard fermentation, but reached the same level by the end of the fermentation.

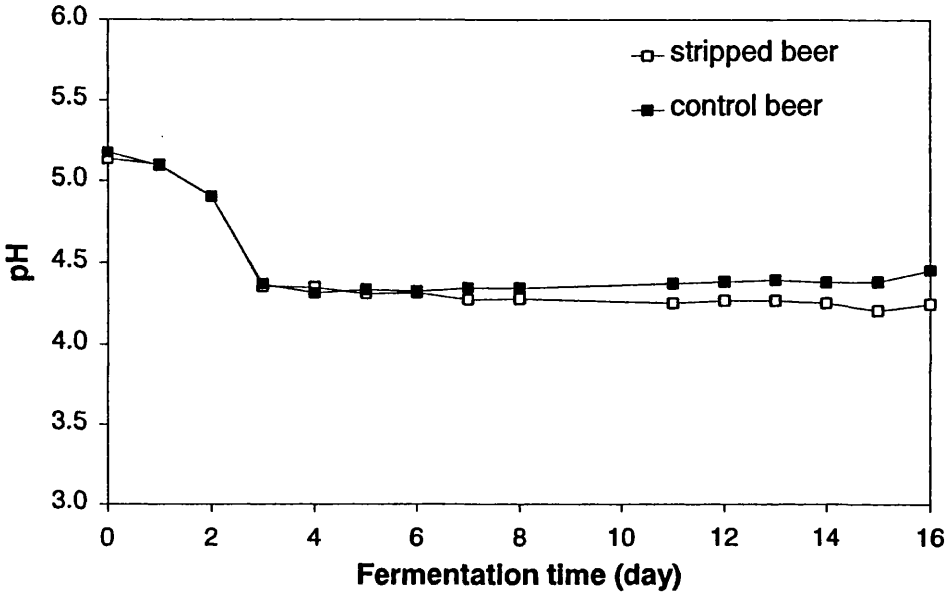

Figure 5–22: Changes in pH in control and stripped medium for Standard 1080 fermentation.

Table 5-14: pH measurements of control and stripped media on day 0 and day 14.

Fermentation set	Initial pH	Final pH		Day at which pH differs between control and stripped medium
		Control	Stripped	
Standard 1080[1]	5.1	4.4	4.2	6-7
Intermittent stripping 1080[2]	5	4	3.9	6-8
Standard 1100[1]	5.1	4.3	4.2	6-7
Non-agitated 1100[1]	5.1	4.4	4.2	8-9
Non-aerated 1100[1]	5.1	4.4	4.3	6-7

[1]carried out with NCYC 1236 at 16°C, [2]carried out with a Wine yeast at 22°C

5.1.8 Effect of stripping on yeast physiology and morphology

5.1.8.1 Suspended cell counts

A typical batch growth curve for yeast can be subdivided into five phases namely lag phase, accelerating growth phase, exponential phase, decelerating growth phase and stationary phase. Due to sampling being carried out daily, it was not possible to follow in detail the lag phase, the accelerating growth phase and the phase of decelerating growth, which are phases occurring within hours rather than days. However, the exponential phase (between day 0 and day 3), where growth increased at a constant rate, and the stationary phase, where cell population had reached its maximum were well defined. The cessation of growth may be due to the depletion of essential nutrients in the medium (i.e. sugars) and/or accumulation of some autotoxic product of yeast (i.e. ethanol) in the medium. By day 3, all fermentations entered the stationary phase. Stripping was initiated after completion of day 3, so that it would not affect the exponential phase of yeast growth.

Only changes in suspended cells counts for Standard 1100 fermentation are shown (Figure 5–23), as the same changes were observed in the other fermentation sets. In control fermentations, the number of suspended cells in the beer medium increased until day 3-4, and started to decline when cells entered the stationary phase. When the fermentation medium was not mechanically agitated, the number of suspended cells declined more than in the standard control medium. In unagitated cultures, biomass concentration may decline as cells flocculate, die and autolyse after a period of stationary phase.

As stripping was only started after day 3 in stripped fermentations, the number of suspended cells in the medium during the exponential phase increased at the same rate as for the control fermentations. When stripping was started, the number of stripped cells increased whereas the number of control cells declined. By the end of the fermentation, the number of stripped cells in suspension was always greater than the number of control cells. The same effect was observed in all the fermentations sets. Results are summarised in Table 5-15. Cell number at day 0 was determined just after the inoculum was introduced into the cool beer wort. The pitching rate or amount of yeast present at the beginning of fermentation varied slightly from

1.1x10E6 to 1.6x10E6 in the fermentations using the ale strain. Because of the counting method which resulted in an average error of 20%, the values of pitching rates were therefore not significantly different. The number of suspended cells on day 3 (end of the exponential phase or beginning of the stationary phase) was between 34 and 43 times more than the initial number of cells. Again, the difference between the different fermentations could be due to the error from the counting method. This was equivalent to about 5 or 6 doublings of the yeast population. The number of cells in control fermentations declined to approximately 1.2-1.9 times the number on day 3, whereas the number of stripped cells increased to about 1.3 times the number on day 3. As shown in Figure 5–23, the number of suspended cells when corrected for the condensate loss was still higher than in control cells. This was true for all the fermentation sets. The number of suspended cells was also measured on the last day of the fermentation, after vigorous mixing of the medium to enable suspension of any sedimented cells. The same results, that is the number of stripped cells was higher than the number of control cell was found.

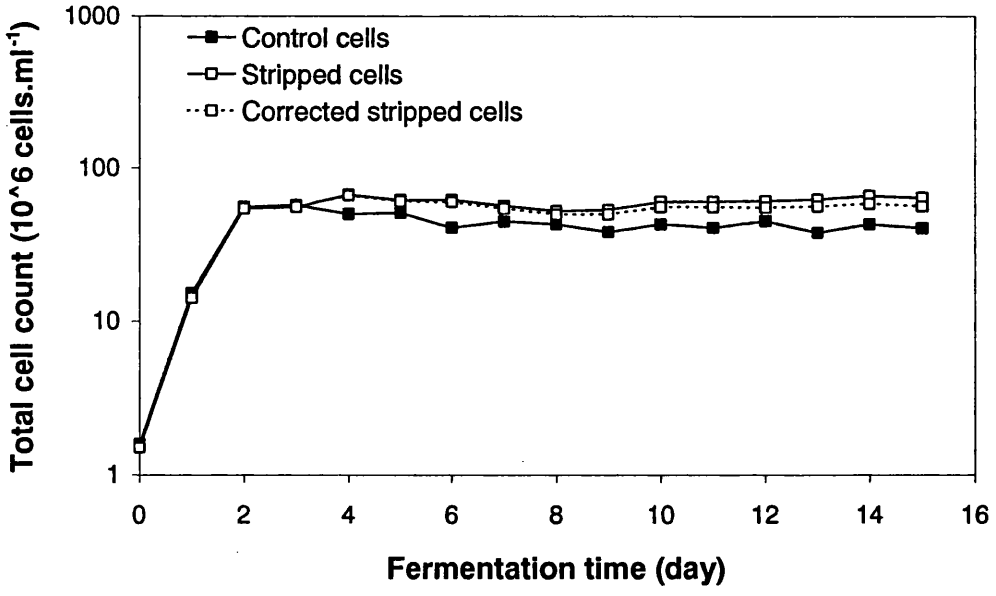

Figure 5–23: Changes in total cell count in control and stripped medium during Standard 1100 fermentation.

Table 5-15: Number of suspended cells in control and stripped fermentations. x_0, x_3 and x_{14} correspond to the number of cells on day 0, day 3 and day 14 respectively.

Fermentation set	Suspended cell number (cells/ml)					Fold increase or decrease of suspended cells				
	x_0	x_3	x_{14} control	x_{14} stripped	x_{14} corrected stripped*	x_3/ x_0	x_3/ x_{14} control	x_{14}/ x_3 stripped	x_{14}/ x_0 control	x_{14}/ x_0 stripped
Standard 1080	1.2x10E6	N/A	2.5x10E7	4.2x10E7	N/A	N/A	N/A	N/A	21	35
Standard 1100	1.6x10E6	5.5x10E7	4.3x10E7	6.6x10E7	5.9x10E7	34	1.3	1.2	27	41
Non agitated 1100	1.1x10E6	4.7x10E7	2.3x10E7	5.1x10E7	4.7x10E7	43	2.0	1.1	21	46
Non aerated 1100	1.6x10E6	6.9x10E7	4.2x10E7	7.7x10E7	7.0x10E7	43	1.6	1.1	26	48

* Number of stripped cells on day 14, corrected for the volume loss due to the removal of the condensate

5.1.8.2 Cell viability

Suspended cells in the beer medium were assessed for viability. Figure 5–24, Figure 5–25 and Figure 5–26 show the changes in cell viability for various fermentations. A decrease in cell viability, as a function of fermentation time, was observed in both control and stripped fermentations. The decrease in cell viability in the stripped medium was less pronounced than the decrease in the control medium.

Results summarised in Table 5-16 show the viability of the initial and final cells in the different fermentation sets. Stripping resulted in between 20 and 51% increase in cell viability compared to the control fermentation at the end of the fermentation. In both standard fermentations with OG 1080 and OG 1100, 80% of stripped cells were still viable at the end of the fermentation, compared to only 62-64 % of control cells. Deviation from those values was observed when the fermentation was not mechanically agitated or initially aerated. Up to 97% and 90% of cells were still viable in the stripped medium when fermentation medium was not agitated and not aerated respectively. Yeast cells from non-aerated control fermentation were also more viable than in the control standard fermentation (75% viable compared to 64%).

The number of budding cells was also followed through the fermentations. In general, a higher number of budding cells was present in the stripped fermentations compared to the control fermentations. In the non-aerated fermentation with OG 1100, 20% of stripped cells were still budding compared to only 10% in the control medium.

Table 5-16: Cell viability in control and stripped fermentations on day 0 and day 14 (NCYC 1236, 16°C).

Fermentation set	Initial viability (%)	Final viability (%)		% increase
		Control	Stripped	
Standard 1080	100	64	80	25
Standard 1100	99	62	80	29
Non-agitated 1100	100	64	97	51
Non-aerated 1100	98	75	90	20

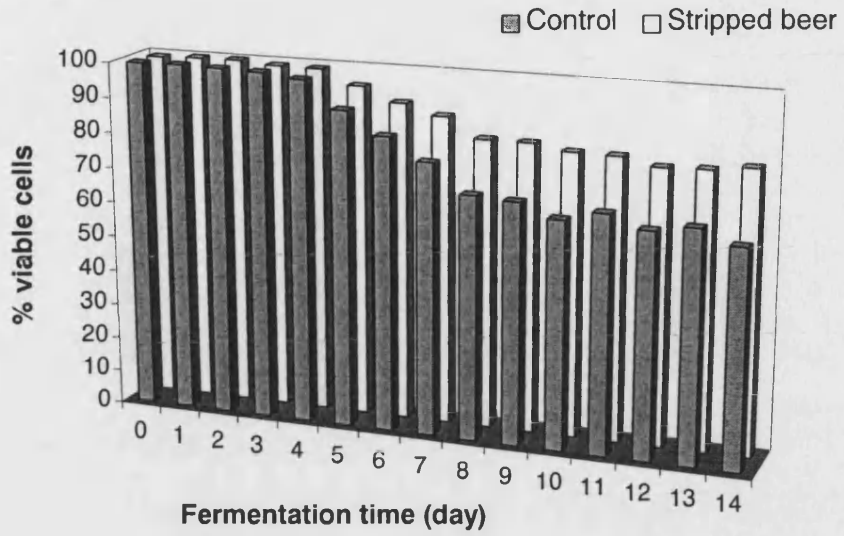

Figure 5–24: Changes in yeast cell viability in control and stripped medium during Standard 1100 fermentation.

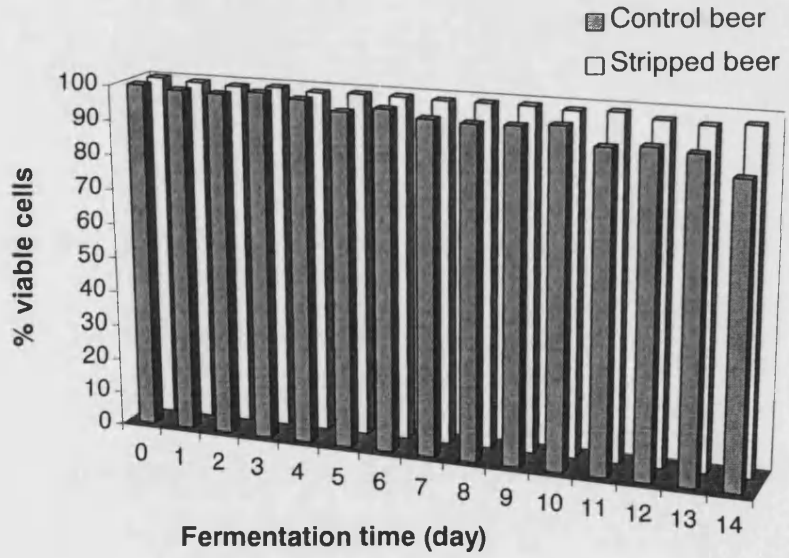

Figure 5–25: Change in yeast cell viability in control and stripped medium during Non-agitated 1100 fermentation.

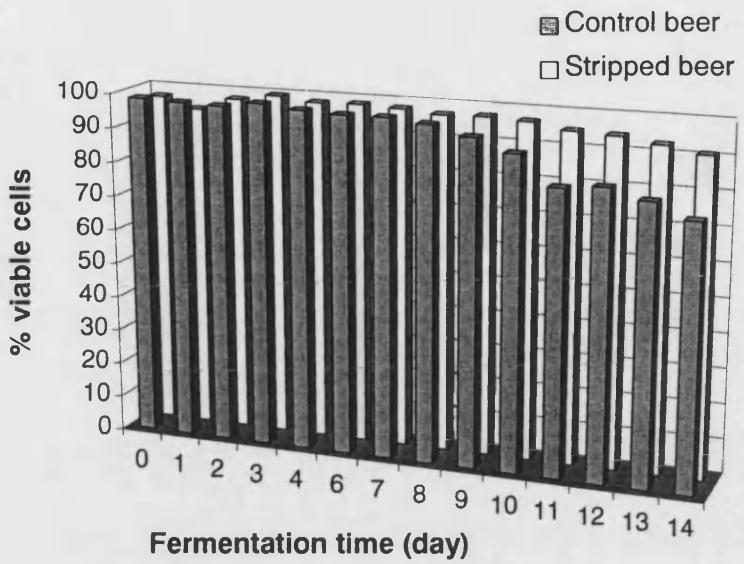

Figure 5–26: Changes in yeast cell viability in control and stripped medium during Non-aerated 1100 fermentation.

5.1.8.3 Cell size

Data on cell size distribution for the highest gravity fermentations with OG 1100 are shown in this section. Similar results were obtained for all the different fermentation sets. It was found that the size distribution of the stripped cells shifted towards a greater number of larger cells by the end of the fermentation. On the other hand, the size distribution of the control cells was not greatly affected during the fermentation. At worst, there was a slight shift towards larger cells, but not to the same extent as observed with the stripped cells. In other words, cells in stripped fermentations tended to get larger than in control fermentations. This phenomenon was also observed microscopically (section 5.1.8.5). Figure 5–27 and Figure 5–28 show the changes in cell size distribution during the Standard 1100 fermentation, in control and stripped media respectively. It was evident from Figure 5–28 (stripped cells) that there was a net shift towards a greater number of larger cells on day 14 and 16. Cell population was the largest within the range 5.8-7.0 mm on day 0, whereas it moved to the range 7.0-8.5 mm on day 14. Even if there was a broadening of the cell size distribution in the control fermentation (Figure 5–27), cell population was the largest within the range 5.8-7.0 at both the beginning and the end of the fermentation.

Similar results were found for the non-agitated fermentation with OG 1100. In Figure 5–30, the size distribution of the stripped cells shift towards a greater number of stripped cells towards the end of the fermentation, whereas in Figure 5–29, only smaller changes occurred in control fermentations.

For the non-aerated fermentation (Figure 5–31), cells in both control and stripped medium on day 14 were compared to the cells in the starter culture on day 0. Again, cell size distribution in the stripped medium was shifted towards a greater number of larger cells, whereas cell size distribution in the control medium became only wider compared to the one in the starter culture.

Cell size analyses were carried out on samples of beer medium containing suspended cells. It, therefore, did not represent the true cell size distribution of whole the cells contained in the fermenters as cells have a tendency of settling at the base of the fermenter. As stripping creates a greater agitation of the medium than the mechanical agitation, it was anticipated that there would be a greater number of cells in suspension in the stripped medium than in the control medium. To check if there was a difference between the settled and suspended cells, deposited cells were resuspended in the beer medium by vigorous agitation of the beer medium on the last day of the fermentation. Figure 5–32 shows that in Standard 1100 fermentation, there was no significant difference in the size distributions of suspended and mixed cells for the control and stripped fermentations.

However, in Non-agitated 1100 fermentation (Figure 5–33), a slight difference was observed when compares the suspended and mixed cells of the control medium, whereas no difference was observed for the stripped cells. The size distribution of the mixed cells from the control medium shifted more towards larger cells than did the size distribution of the suspended cells. This was expected as more cells flocculated when the medium was not mechanically agitated. This effect was confirmed in Figure 5–34 which presents a comparison of the mixed and suspended cells of the control medium between Non-agitated 1100 fermentation and Standard 1100 fermentation.

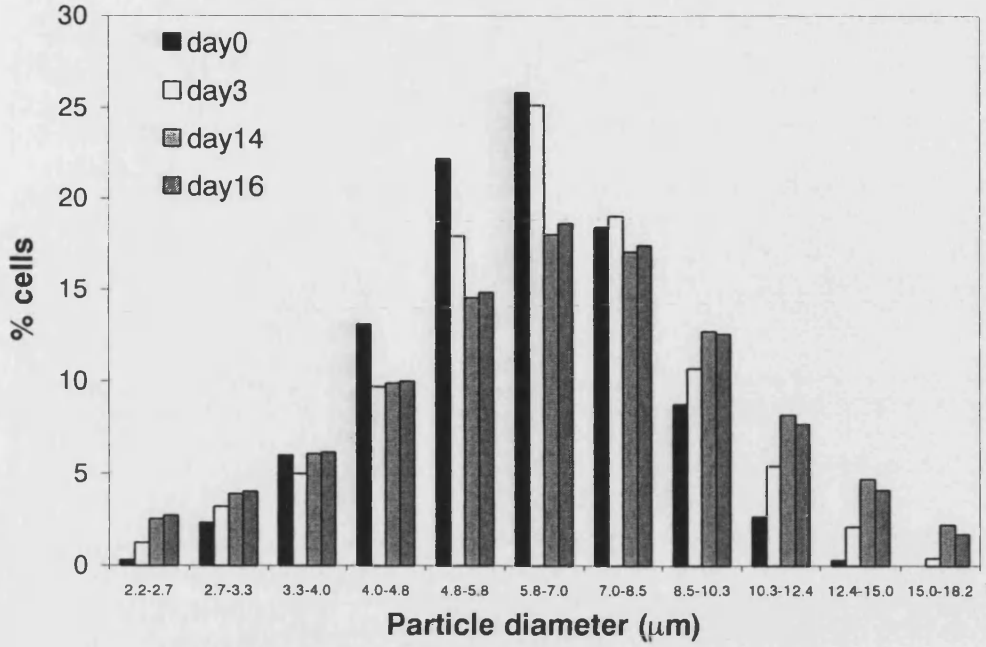

Figure 5–27: Changes in cell size distribution in control medium during Standard 1100 fermentation.

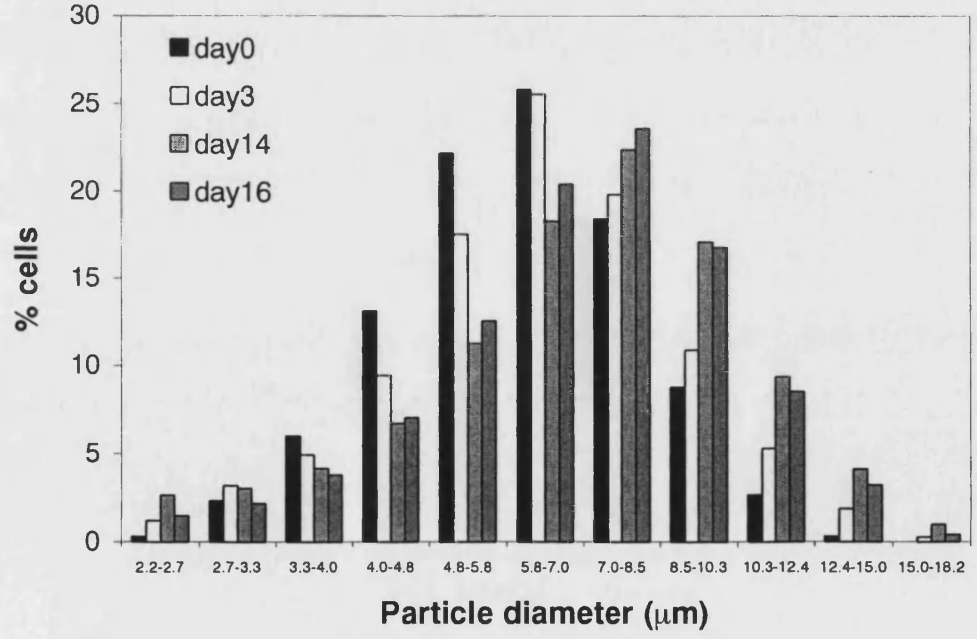

Figure 5–28: Changes in cell size distribution in stripped medium during Standard 1100 fermentation.

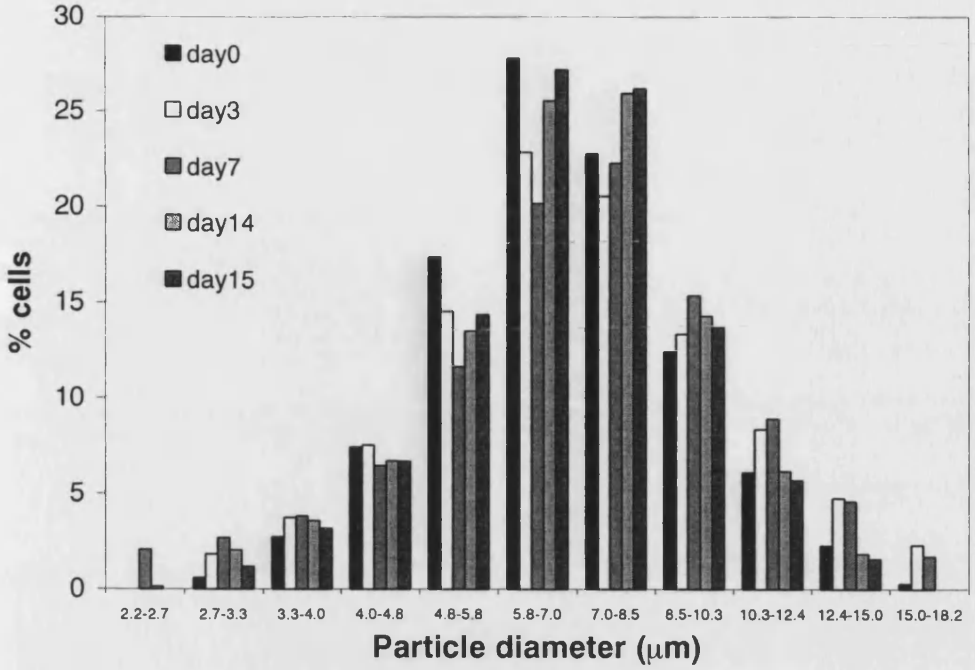

Figure 5–29: Changes in cell size distribution in control medium during Non-Agitated 1100 fermentation.

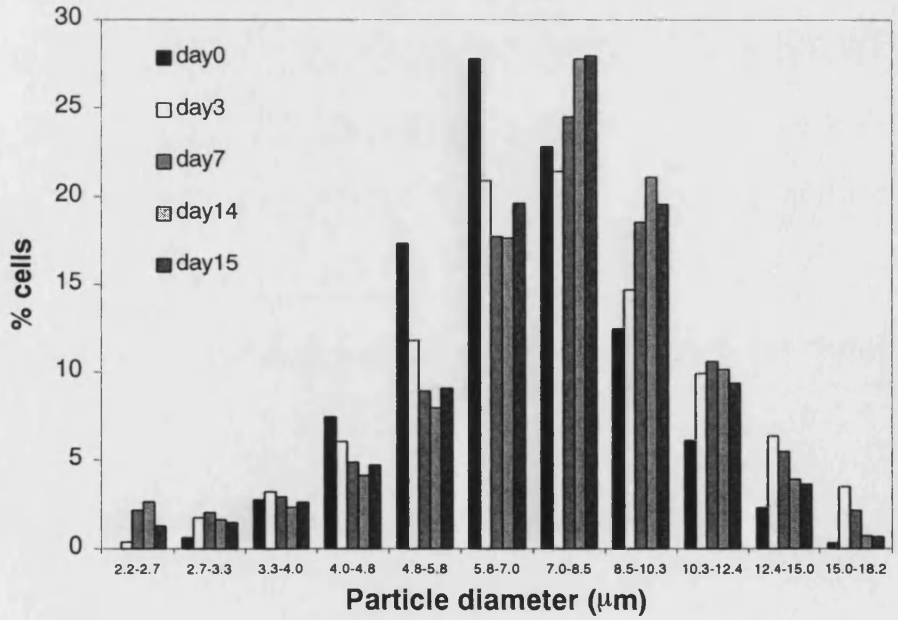

Figure 5–30: Changes in cell size distribution in stripped medium during Non-Agitated 1100 fermentation.

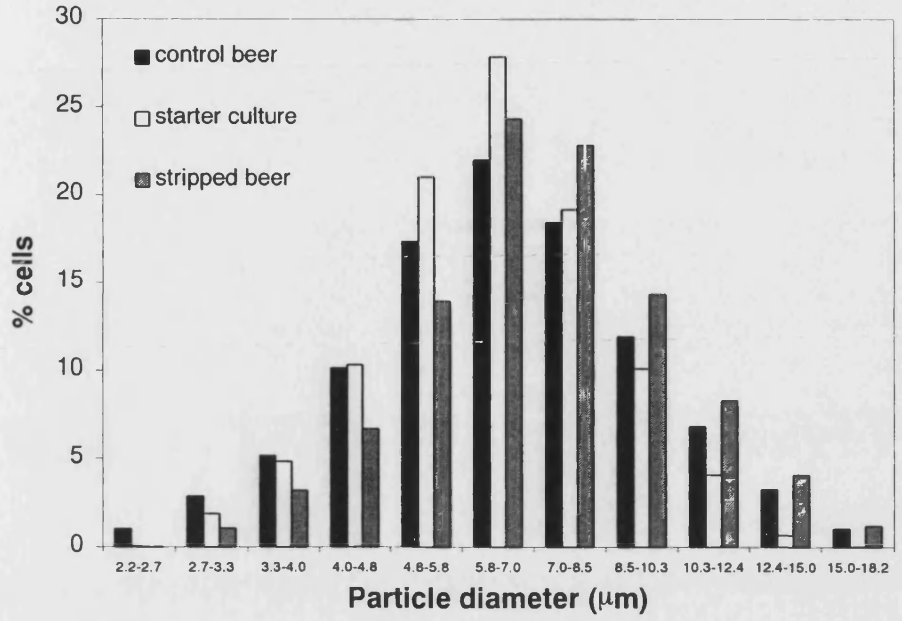

Figure 5–31: Comparison of the cell size distribution between the starter culture, the control medium and the stripped medium in Non-aerated 1100 fermentation.

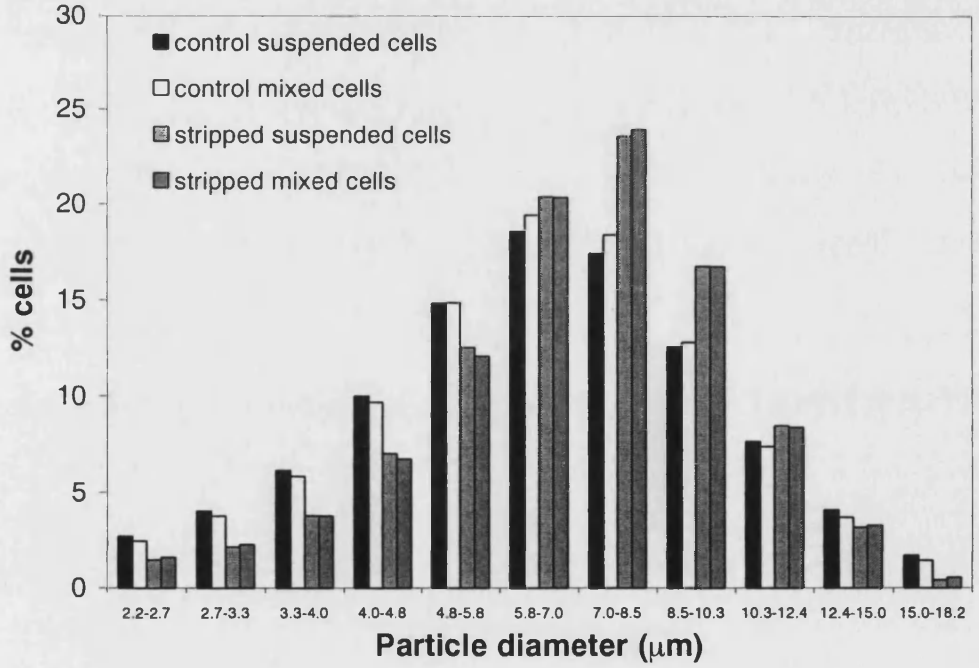

Figure 5–32: Comparison between suspended cells and mixed cells at the end of the fermentation (day16) in Standard 1100 fermentation.

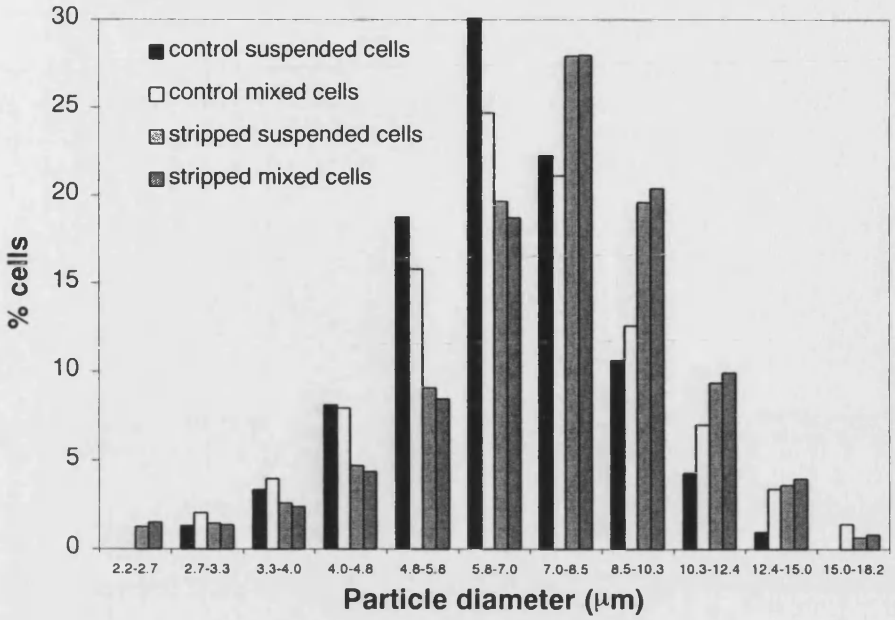

Figure 5–33: Comparison between suspended cells and mixed cells in control and stripped medium in Non-agitated 1100 fermentation at the end of the fermentation.

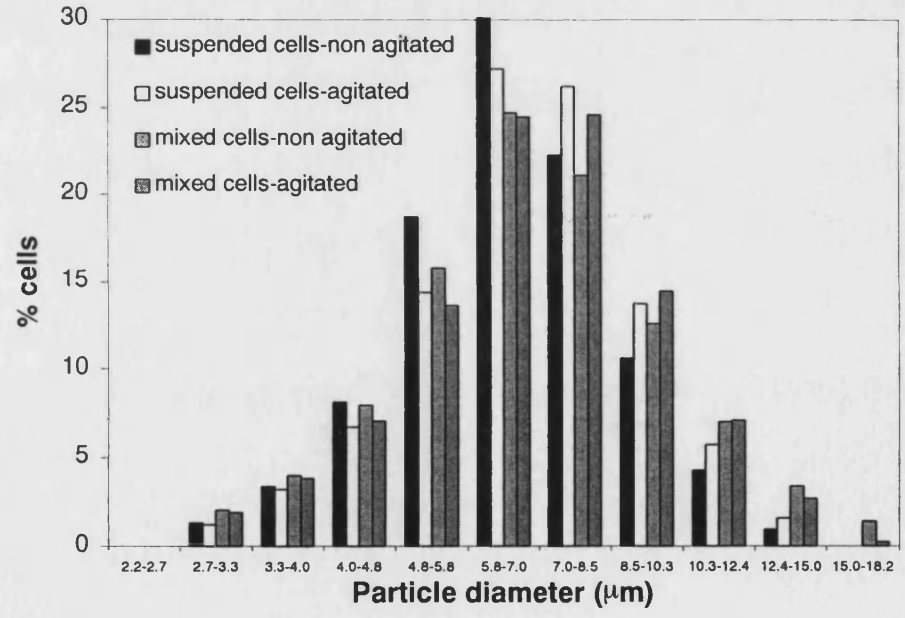

Figure 5–34: Comparison of cell size distributions between Standard 1100 and Non-agitated 1100 in control media at the end of the fermentation.

5.1.8.4 Cell biomass

To determine whether the increase in cell size observed during stripped fermentations was due to accumulation of matter in the cell or to simple plasmolysis phenomenon, dry cell weight was measured as described in Chapter 3, and related to the number of cells.

Biomass was measured in Standard 1100 and in Non-agitated 1100 fermentation. As expected, biomass production measured by dry weight followed the same changes as the ones found for the suspended cells during the fermentation (Figure 5–35). An apparent increase in biomass production was observed in the stripped medium, whereas an apparent decrease in biomass production was observed in the control medium. Figure 5–36 shows the change in dry cell mass as measured in $g.cell^{-1}$. Dry cell mass started to increase in the stripped medium compared to the control medium after day 6. The difference between the stripped cells and the control cells was reduced towards the end of the fermentation, which seemed to be inconsistent. The extent of the decrease and the increase in the number of suspended-stripped cells was questionable, and probably contributed to the inconsistency of the results found for the dry cell mass. Indeed, if the decrease in the number of suspended stripped cells (a minimum number of cells was observed around day 8) was only due to experimental error, dry cell mass would then be very similar in both control and stripped fermentations.

However, an increase in dry cell mass was also observed in the stripped medium of the non-agitated fermentation after day 5. No decrease (to the extent found in the stripped medium of Standard 1100 fermentation) in the number of suspended stripped cell number was measured after day 5. Therefore, the increase in dry cell mass in the stripped medium compared to the control medium was probably a true result.

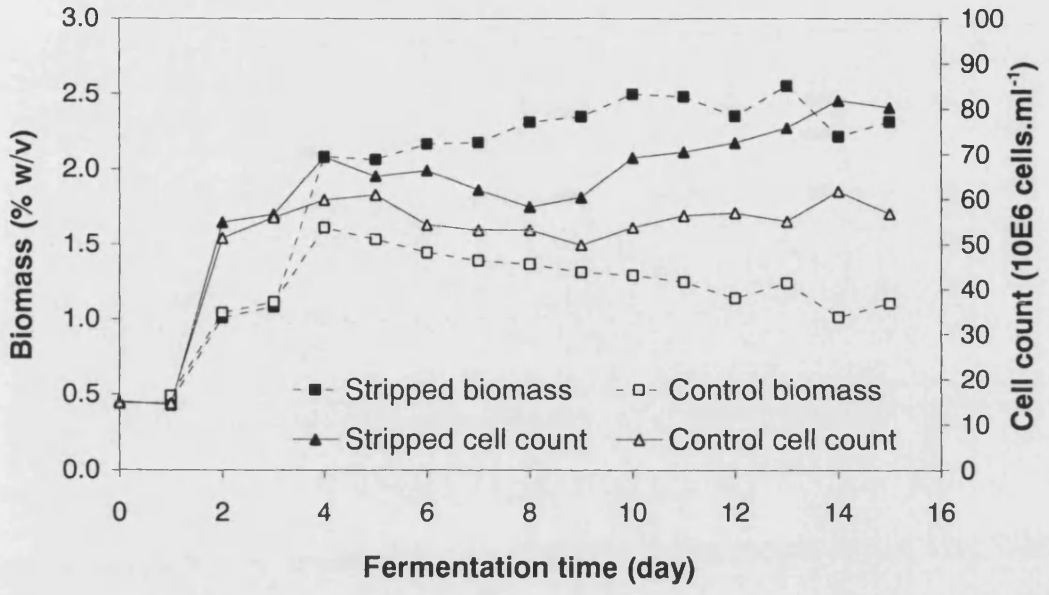

Figure 5–35: Biomass and suspended cell counts in control and stripped medium during Standard 1100 fermentation.

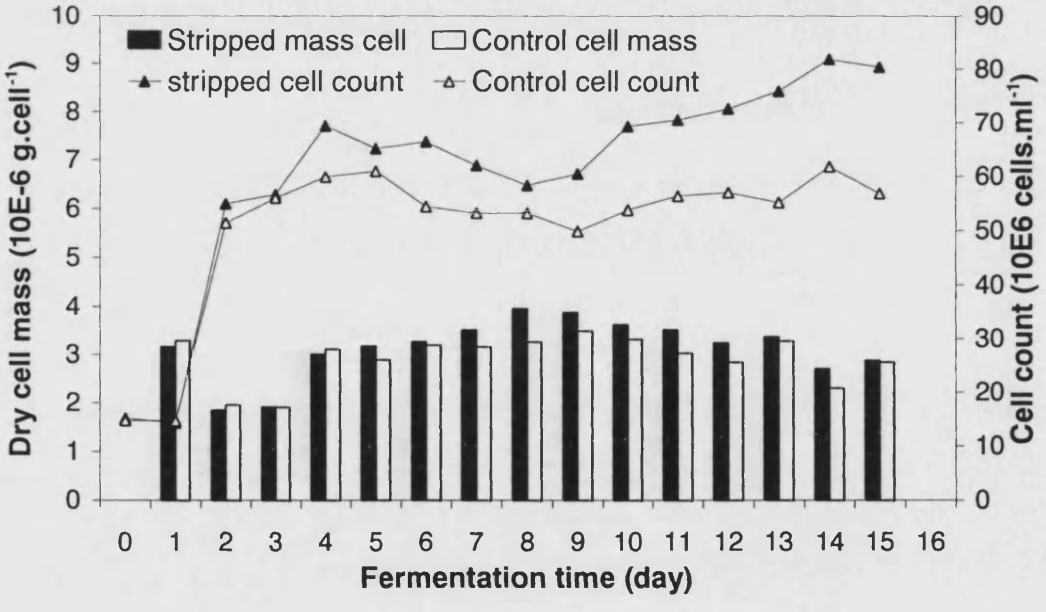

Figure 5–36: Dry cell mass and suspended cell counts in control and stripped medium during Standard 1100 fermentation.

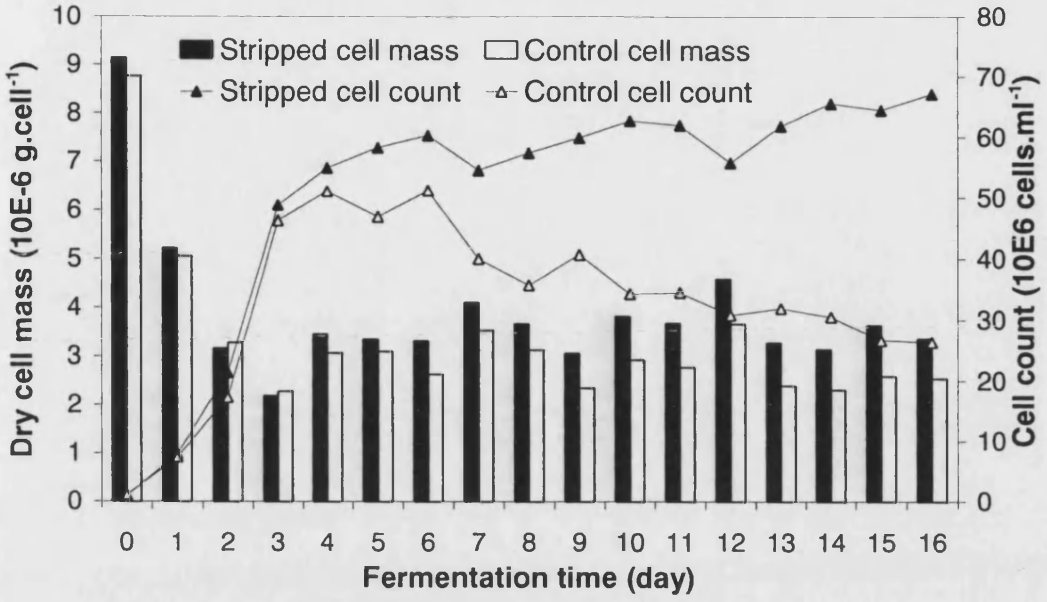

Figure 5–37: Dry cell mass and suspended cell counts in control and stripped media during Non-agitated 1100 fermentation.

5.1.8.5 Cell morphology

Cell morphology was followed up by optical microscopy and Scanning Electron Micrography (SEM). The objective was to discover if stripping caused a change in the shape of the yeast cells. As Huxtable [1993] found some differences in cell morphology when comparing stripped yeast cells with control cells, a careful examination of the cells was carried out. The cell morphology was observed at different times during the fermentation in the various set of fermentations. Similar results were found for all the sets. Figure 5–37 shows an optical microscope picture of the yeast cells on day 3 just before initiating gas stripping. The ovoidal shape of the *Saccharomyces* was well defined. As the cells were still in the exponential phase where cell proliferation occurs, most of the cells were still budding. After 14 days of fermentation, the cells became rounder losing slightly their ovoidal shape. This phenomenon was more pronounced for the stripped cells (Figure 5–39), and was attributable to increased osmotic pressure. When comparing Figure 5–40 to Figure 5–39, it was evident that the stripped cells were on average larger than control cells, which has been demonstrated by the distribution in cell size (section 5.1.8.3). There was no discernible difference in the shape of the cells except that, stripped cells due

to their larger volume tend to be even more rounder than control cells. In rare occasions, some elongated cells were observed in both the control and stripped medium, at random time during the fermentation. This slight elongation was not associated with a change of environment as it was found in both control and stripped fermentations. Contrary to this finding, the cell elongation discovered previously by Huxtable [1993] in stripped cider fermentations, was more severe and the cells were described as having a mycelial form.

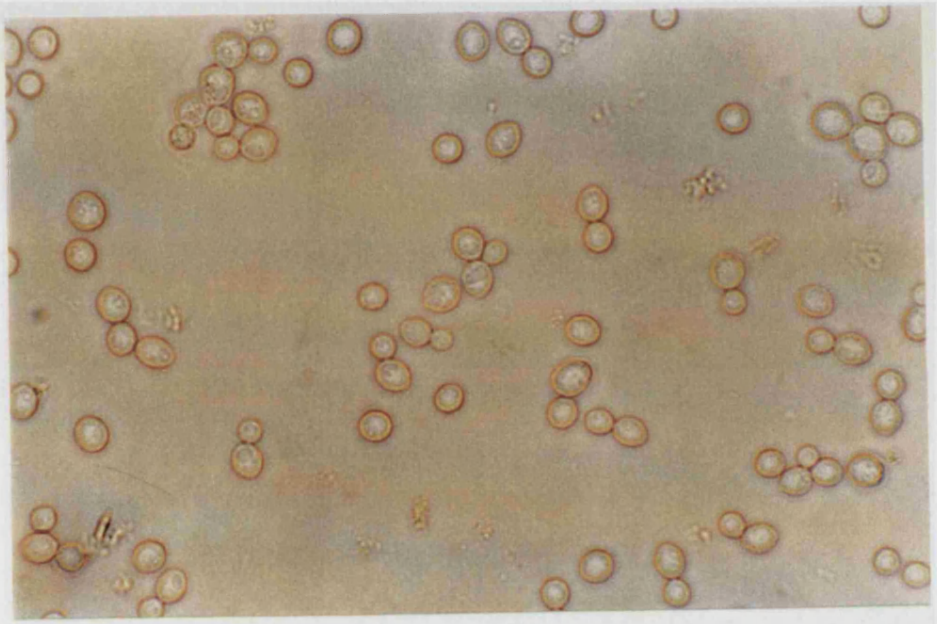

Figure 5–38: Optical microscope picture of yeast cells (NCYC 1236) on day 3 before initiating CO_2 stripping.

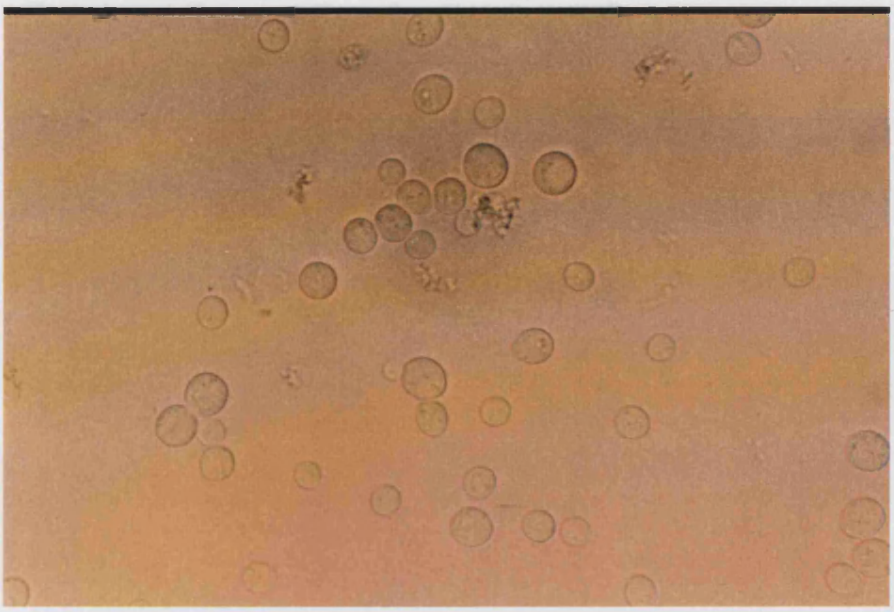

Figure 5–39: Optical microscope picture of stripped yeast cells (NCYC 1236) on day 14 of Standard 1100 fermentation.

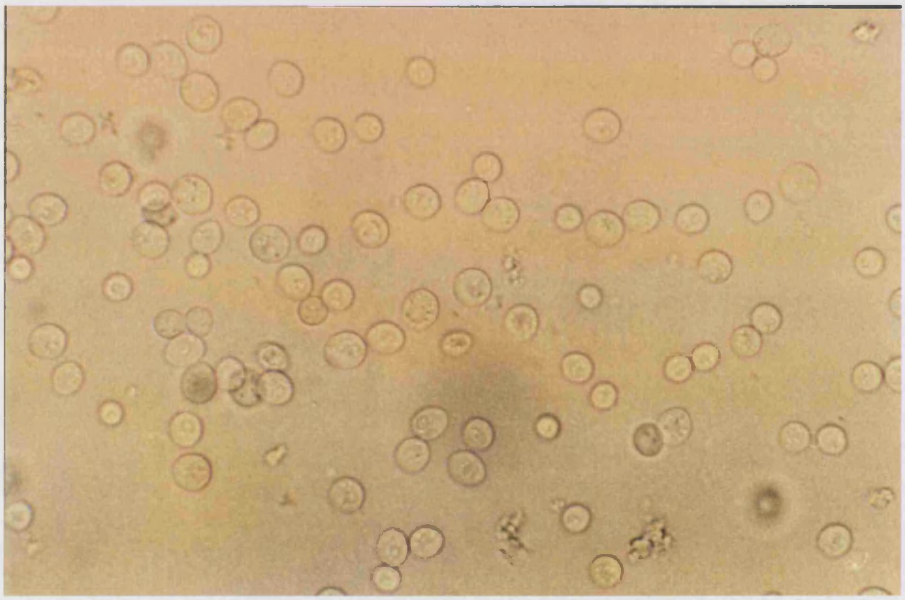

Figure 5–40: Optical microscope picture of control yeast cells (NCYC 1236) on day 14 of Standard 1100 fermentation.

5.1.9 Effect of stripping on the production of beer volatiles other than ethanol

5.1.9.1 Changes in volatile compounds concentration

As described in section 5.1.2.5, only the most predominant volatile compounds, namely isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde were quantified in the beer medium in control and stripped fermentations. Due to a relatively large coefficient of variation on the measured medium concentrations, taking into account absolute values would probably lead to inaccurate interpretation of the results. However, differences between stripped and control concentrations were not within the experimental error and could therefore be interpreted. A pattern of the effect of stripping on the production of volatile compounds in stripped and control fermentations was observed.

Figure 5–41, Figure 5–42, Figure 5–43, Figure 5–44, Figure 5–45 show the changes in isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde in stripped and control medium, and in condensate during Standard 1100 fermentation. For all the compounds, stripping reduced the concentration of the volatile compounds in the medium. The three higher alcohols were reduced by 40-47%, 38-39% and 51-56% in the intermittent stripping 1080, non-aerated 1100 and standard 1080 fermentations respectively (Table 5-17). In the standard 1100 fermentation, however, the three higher alcohols were decreased by 70-72%. While isoamyl alcohol, isobutanol, propanol and acetaldehyde remained in the stripped medium at the end of the fermentation, ethyl acetate was completely removed from the fermentation by day 16 (Figure 5–44). In some cases, ethyl acetate was completely removed from the stripped medium at an early stage in the fermentation. Acetaldehyde was still present at the end the stripped fermentation at approximately 6-7 $mg.L^{-1}$ in both Standard 1100 and Non-aerated 1100 fermentations (it was reduced by 70% in both fermentations). Table 5-19 summarises the results for the different experiments by presenting final concentrations (on day 16 of the fermentation) for each volatile compound in stripped and control fermentations.

The changes in medium and condensate concentration during the fermentation were similar for the alcohols, ethyl acetate and acetaldehyde. Their changes followed quite

closely the changes observed for ethanol. For example, control isoamyl alcohol reached a plateau around day 12, while stripped isoamyl alcohol reached a maximum, before being reduced by further stripping. The reduction of medium isoamyl alcohol concentration after day 12 was also reflected by a decrease in concentration of condensate isoamyl alcohol. Even if the results were not as clear for isobutanol and propanol, similar changes were observed. As observed for medium ethanol in this particular fermentation set (Standard 1100), isoamyl alcohol, isobutanol and propanol were at their highest concentration the day after which stripping was initiated. A peak in condensate concentration around day 10 was also observed for ethyl acetate and acetaldehyde. At the difference with the other volatile compounds, acetaldehyde concentration in the stripped medium was higher than in control medium between day 4 and day 8. This led to the hypothesis that net acetaldehyde production would probably be higher than in control fermentation. After day 8, further stripping led to a decrease in acetaldehyde below control acetaldehyde concentration.

Changes in ethyl acetate were not as clear as for the other beer volatiles, due to ethyl acetates very low condensate concentration being at the limit of detection. However, trap ethyl acetate concentration which was on average 70 times higher than in the condensate (Table 5-29), followed the same pattern of changes during the fermentation. It was therefore possible to discuss the results found for condensate ethyl acetate. Production rate for ethyl acetate was quite high from the start of the fermentation before slowing between day 3 and day 4. Ethyl acetate concentration was then maintained at approximately 40 $mg.L^{-1}$. Condensate ethyl acetate peaked on day 10, as observed for acetaldehyde.

The condensate and trap (when used) constitute a concentrated fraction of the different beer volatiles. Condensate (or trap) to medium ratio are shown in Table 5-18. For example, the higher alcohols were approximately 3 and 15 times more concentrated in the condensate and in the trap respectively than in the medium.

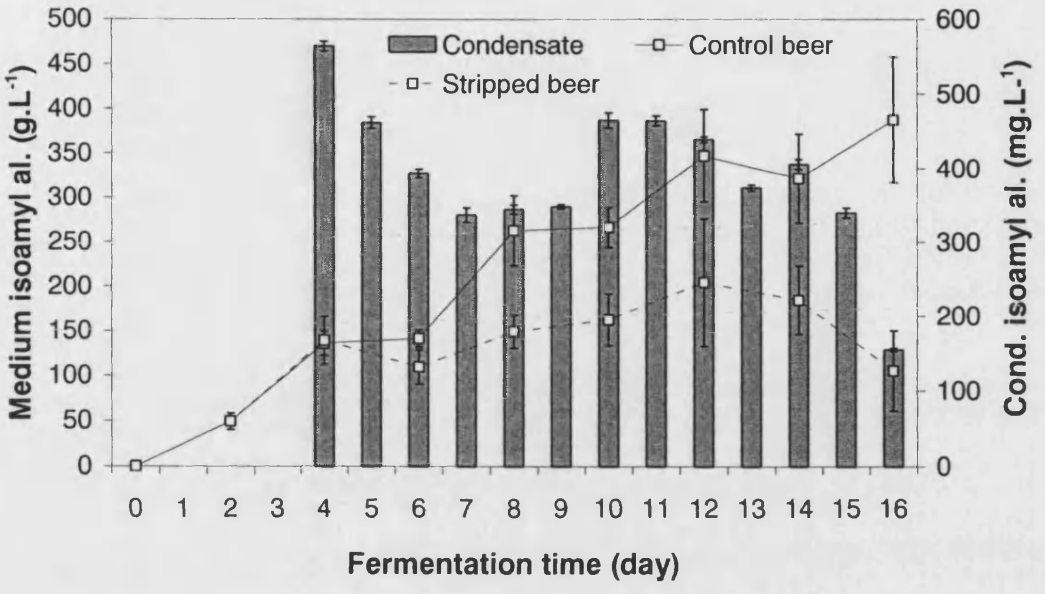

Figure 5–41: Changes in isoamyl alcohol concentration in control and stripped medium during Standard 1100 fermentation.

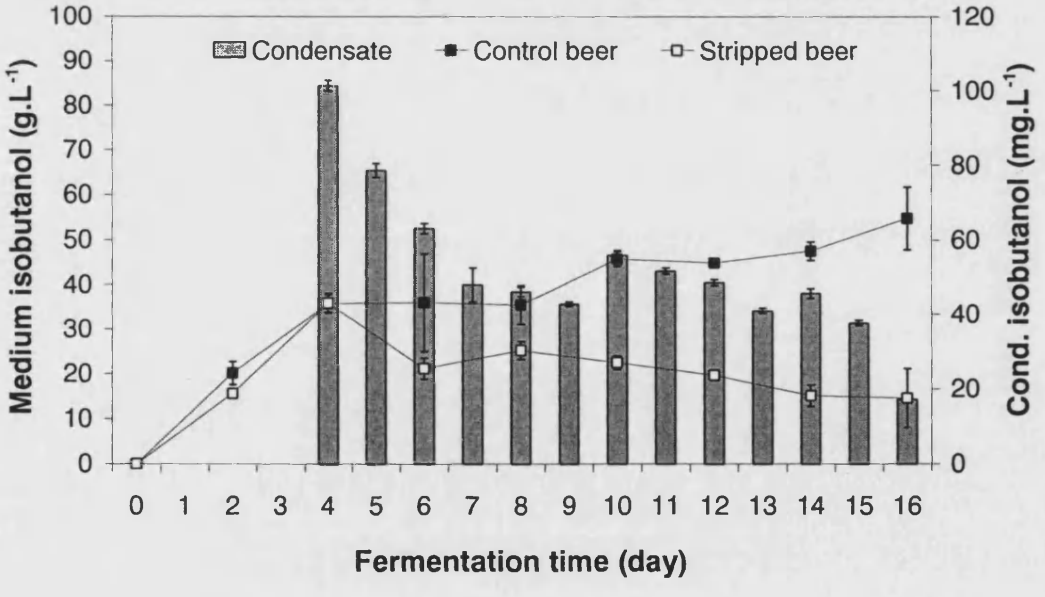

Figure 5–42: Changes in isobutanol concentration in control and stripped medium during Standard 1100 fermentation.

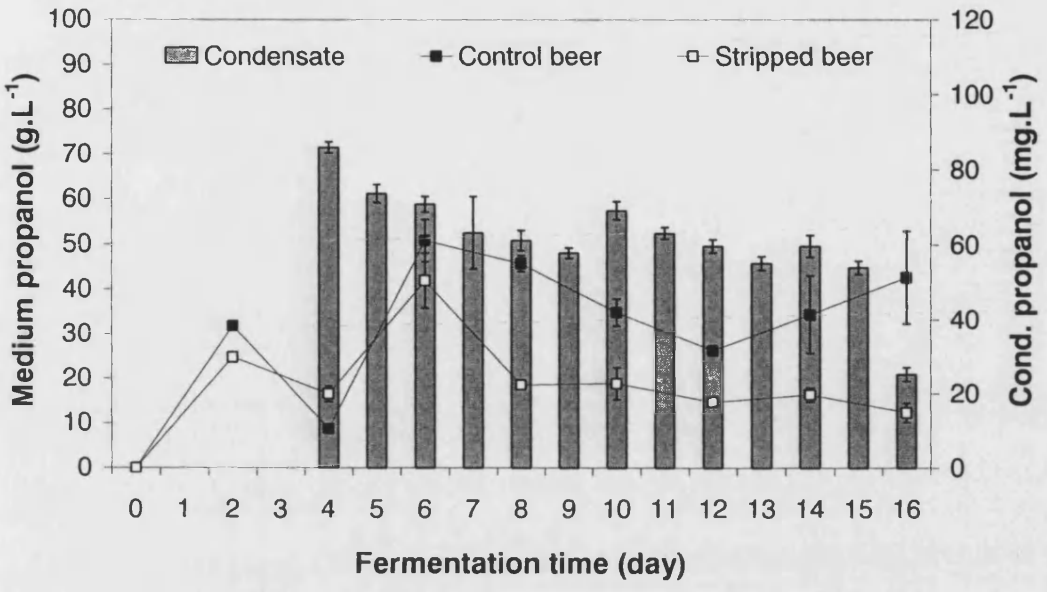

Figure 5–43: Changes in propanol concentration in control and stripped medium during Standard 1100 fermentation.

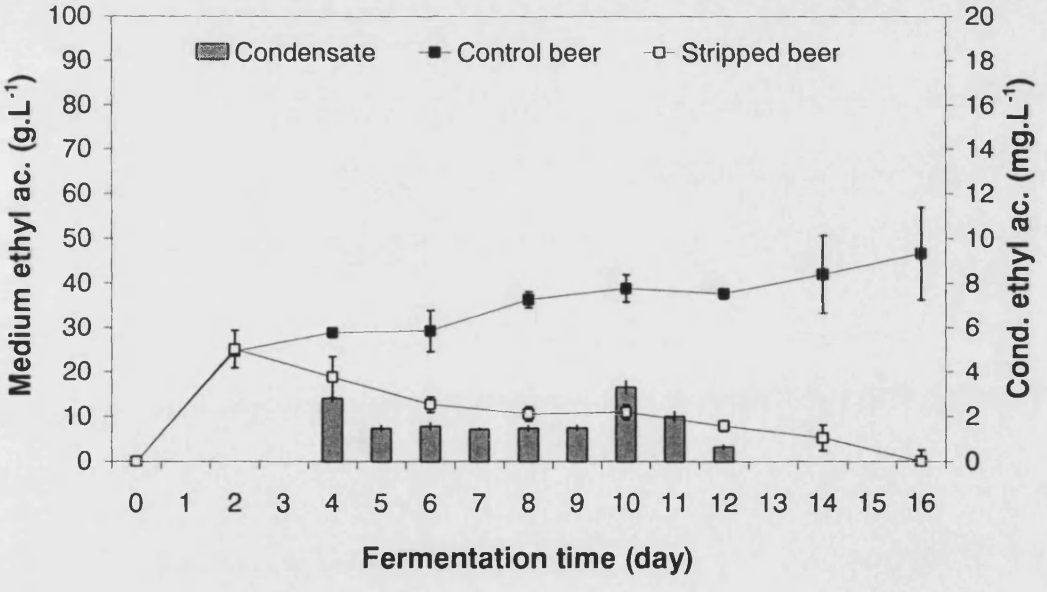

Figure 5–44: Changes in ethyl acetate concentration in control and stripped medium during Standard 1100 fermentation.

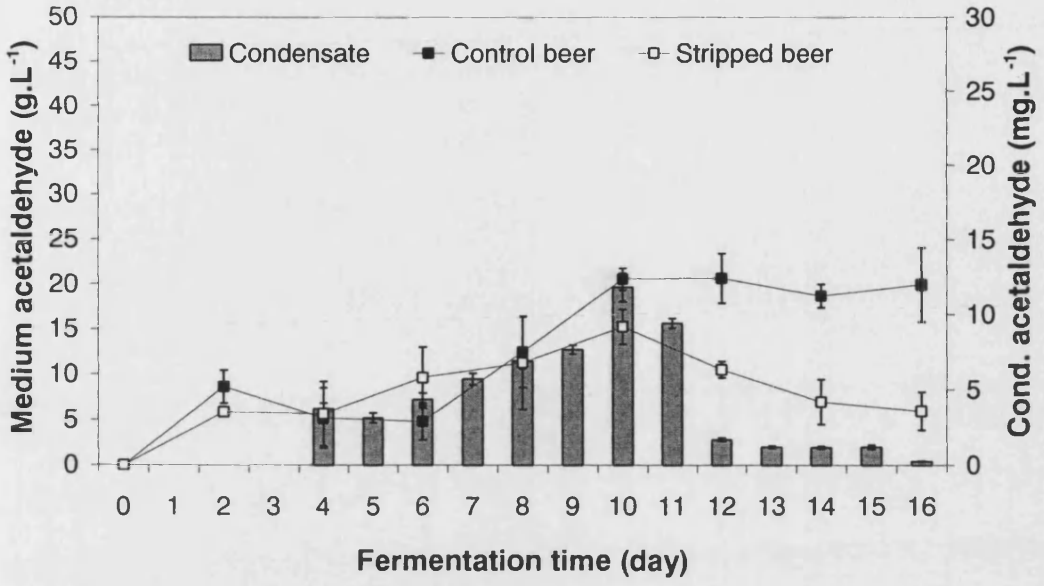

Figure 5–45: Changes in acetaldehyde concentration in control and stripped medium during Standard 1100 fermentation.

Table 5-17: Ratio of volatile compound concentration between stripped medium and control medium, expressed in %.

	Isoamyl alcohol	Isobutanol	Propanol	Acetaldehyde
Standard 1080[1]	56	51	56	N/A
Intermittent stripping 1080[2]	47	40	41	N/A
Standard 1100[1]	70	72	72	70
Non-aerated 1100[1]	39	39	38	70

[1]carried out with NCYC 1236 at 16°C, [2]carried out with a Wine yeast at 22°C.

Table 5-18: Condensate or trap to medium concentration ratio.

	Condensate/medium Concentration ratio			Trap/medium concentration ratio
	Standard 1100	Non-aerated 1100	Standard 1080	Standard 1100
Acetaldehyde	0.4	0.7	0.4	3
Ethyl acetate	0.1	0	0.4	10
Propanol	3.4	3.4	1.1	14
Isobutanol	2.4	3.6	2.1	18
Isoamyl alcohol	2.7	4.5	5.6	17

Table 5-19: Final concentration ($mg.L^{-1}$), on day 16, of selected beer volatile compounds in stripped and control fermentation (without correction for volume loss).

	Isoamyl alcohol		Isobutanol		Propanol		Ethyl acetate		Acetaldehyde	
	Control	Stripped	Control	Stripped	Control	Stripped	Control	Stripped	Control	Stripped
Standard 1080[1]	123	54	61	30	36	16	55	0	N/A	N/A
Intermittent stripping 1080[2]	223	118	50	30	17	10	N/A	N/A	N/A	N/A
Standard 1100[1]	350	106	54	15	43	12	46	0	20	6
Non-aerated 1100[1]	193	118	75	46	32	20	55	0	23	7

[1]carried out with NCYC 1236 at 16°C, [2]carried out with a Wine yeast at 22°C

5.1.9.2 Net production of the main beer volatile compounds

Table 5-20 summarises the results on net production, expressed as the total mass of the volatile compound produced (including condensate and trap for the stripped fermentations) in the stripped medium relative to the control medium. For all the fermentations investigated, the percentage was always below 100%, which shows that for each compound apparent net production in stripped fermentations was lower than in control fermentations. As it was observed in Chapter 4 and in section 5.1.4.3 for ethanol production, the volatile compounds extracted by stripping was only partially captured by the condensation unit used in the experiments. Therefore, the data presented in Table 5-20 can be considered as apparent net production.

The apparent relative mass of the higher alcohols, namely isoamyl alcohol, isobutanol and propanol, produced by the stripped medium was higher (between 56 and 92 %w/w) than the relative mass of acetaldehyde (33-39%) and ethyl acetate (1.2-11.5 %) produced. As the higher alcohols are less volatile than esters and acetaldehyde (Chapter 4), they were more condensed than the esters and acetaldehyde. However, it could also mean that there was a disproportionate production of higher alcohols in the stripped medium compared to ethyl acetate and acetaldehyde. In other words stripping could change the flavour balance by producing less of some compounds (in this case ethyl acetate and acetaldehyde).

Detailed examination of the total mass of each compound collected in the different phases (medium, sample, condensate and trap when used) is presented in Table 5-21 to Table 5-25. The mass of each individual compound was calculated from the concentrations obtained by GC analysis, and from their respective condensate and trap volume. Net production was expressed as $mg.L^{-1}$ of initial fermentation volume. As mentioned above, apparent net production of isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde was lower in the stripped medium than in the control medium for the different fermentation sets.

Table 5-20: Total mass of selected beer volatile compound produced in stripped fermentations relative to control fermentations, expressed in % w/w.

	Isoamyl alcohol	Isobutanol	Propanol	Ethyl acetate	Acetaldehyde
Standard 1080	70	56	90	2.5	N/A
Standard 1100	79	66	73	115	39
Non-aerated 1100	79	75	92	1.2	33

Table 5-21: Total production of isoamyl alcohol ($mg.L^{-1}$ initial volume) in control and stripped fermentations.

		Medium	Sample	Condensate	Trap	Total
Standard 1080	**Control**	123	8	N/A	N/A	131
	Stripped	64	5	23	N/A	92
Standard 1100	**Control**	319	24	N/A	N/A	343
	Stripped	116	14	29	113	272
Non-aerated 1100	**Control**	169	21	N/A	N/A	191
	Stripped	91	17	60	N/A	169

Table 5-22: Total production of isobutanol ($mg.L^{-1}$ initial volume) in control and stripped fermentations.

		Medium	Sample	Condensate	Trap	Total
Standard 1080	**Control**	56	5	N/A	N/A	61
	Stripped	24	4	7	N/A	34
Standard 1100	**Control**	49	4	N/A	N/A	53
	Stripped	12	2	4	18	35
Non-aerated 1100	**Control**	72	8	N/A	N/A	81
	Stripped	36	7	18	N/A	61

Table 5-23: Total production of propanol ($mg.L^{-1}$ initial volume) in control and stripped fermentations.

	Medium	Medium	sample	Condensate	Trap	Total
Standard 1080	**Control**	34	4	N/A	N/A	38
	Stripped	28	4	3	N/A	35
Standard 1100	**Control**	38	3	N/A	N/A	42
	Stripped	13	2	4	12	31
Non-aerated 1100	**Control**	23	3	N/A	N/A	26
	Stripped	16	2	6	N/A	24

Table 5-24: Total production of ethyl acetate ($mg.L^{-1}$ initial volume) in control and stripped fermentations.

		Medium	Sample	Condensate	Trap	Total
Standard 1080	**Control**	53.6	5.3	N/A	N/A	58.9
	Stripped	0.0	1.3	0.2	N/A	1.5
Standard 1100	**Control**	41.7	3.5	N/A	N/A	45.2
	Stripped	0.0	1.0	0.1	4.1	5.2
Non-aerated 1100	**Control**	53.6	5.3	N/A	N/A	58.9
	Stripped	0.0	0.5	0.2	N/A	0.7

Table 5-25: Total production of acetaldehyde ($mg.L^{-1}$ initial volume) in control and stripped fermentations.

		Medium	Sample	Condensate	Trap	Total
Standard 1080	**Control**	N/A	N/A	N/A	N/A	N/A
	Stripped	N/A	N/A	N/A	N/A	N/A
Standard 1100	**Control**	17.9	1.3	N/A	N/A	19.2
	Stripped	4.5	0.8	0.4	1.6	7.3
Non-aerated 1100	**Control**	20.8	1.3	N/A	N/A	22.2
	Stripped	5.9	0.9	0.4	N/A	7.3

5.1.10 Corrected ethanol and other beer volatiles production using UNIFAC calculations

From Chapter 4, it was found that total ethanol stripped from a synthetic medium was not fully recovered by the condensation unit, and that only 24% of the ethanol was recovered. It was therefore anticipated that measured ethanol production was not representative of the true ethanol production. The percentage recovery, estimated in Chapter 4 was used to predict the true ethanol production in stripped fermentations. Table 5-26 compares measured ethanol production with maximum theoretical ethanol production (from complete conversion of fermented sugars into ethanol), and with corrected ethanol concentration using UNIFAC estimation. Corrected ethanol production with UNIFAC estimation was 29% higher than the maximum theoretical ethanol. This shows that UNIFAC estimation, which is accurate for synthetic mixtures containing ethanol and other volatile compounds, is not directly applicable to real mixtures such as beer fermentation medium.

Estimated percentage recovery (Chapter 4) of the other main beer volatiles (isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde) was also used in order to estimate the "true" production of these compounds in the stripped medium of Standard 1100 fermentation. The calculations are presented in Table 5-27. Percentage recoveries were estimated using Kvalues and extrapolated Henry's constant (Chapter 4). Due to their similarities, an average between the two was calculated. Using the estimated percentage recovery, it was found that stripping resulted in a 1.5, 1.8 and 1.6 fold increase in isoamyl alcohol, isobutanol and propanol production respectively. For ethyl acetate, however, the two methods (Kvalues and extrapolated Henry's constant), resulted in two different estimation of the volatility. The two different values of percentage recovery resulted in either a 2-fold increase or a 2.4-fold decrease of production. With acetaldehyde, stripping resulted in a 6-fold increase in net production, and a resulting concentration of 111 $mg.L^{-1}$. At this concentration in beer, acetaldehyde could be perceived as an off-flavour. The estimation using percentage recovery only provides an approximate estimation of the volatile production, as many assumptions were drawn.

Table 5-26: Comparison between measured ethanol production with maximum theoretical ethanol production and corrected ethanol production using UNIFAC estimation (after 14 days of fermentation).

	Measured Ethanol production	Maximum theoretical ethanol	Corrected ethanol using UNIFAC*	%increase using UNIFAC
	$g.L^{-1}$ initial volume			
Standard 1080	40	72	N/A	N/A
Standard 1100	55	85	109	28
Non-agitated 1100	47	73	95	30
Non-aerated 1100	48	85	N/A	N/A

*UNIFAC estimation (Chapter 4) of ethanol loss was calculated for systems using both condensate (0°C) and trap (-40°C). In Standard 1080 and Non-aerated 1100 fermentations, a condensate only was used to collect the extracted volatile compounds. Therefore, corrected ethanol using UNIFAC is not presented for those fermentations.

Table 5-27: Estimation of true beer volatiles production using percentage recovery as calculated in Chapter 4.

	Isoamyl alcohol	Isobutanol	Propanol	Ethyl acetate	Acetaldehyde
Percentage recovery[1]	27	21	24	3.6 (a/K) **17.9 (a/H)**	1.5
Measured cond&trap ($mg.L^{-1}$initial vol)	142	22	16	4.2	2
Corrected condensate and trap ($mg.L^{-1}$) [2]	523	107	67	117 **(24)**	138
Corrected condensate and trap ($mg.L^{-1}$) [3]	402	82	52	90 **(18)**	106
Corrected stripped medium ($mg.L^{-1}$)	532	96	67	90 **(19)**	111
Control medium ($mg.L^{-1}$)	343	53	42	45.2	19.2
Fold increase[4]	1.5	1.8	1.6	2 **(-2.4)**	6

[1] As calculated in Chapter 4.
[2] Correction using percentage recovery.
[3] Additional correction using the 29% difference found between predicted ethanol (UNIFAC) and theoretical ethanol (Table 5-26).
[3] Fold-increase in volatile production between control medium and stripped medium

5.1.11 Volatile compounds profile of condensate and trap

Due to the more complex analytical method needed to analyse chemical compounds in heterogeneous systems such as fermentations medium, only the major beer volatile compounds were tentatively quantified in the fermentation media, as discussed in section 5.1.9.1. On the other hand, condensate and trap fractions were easier to analyse, due to the relative simplicity of the solution containing pure chemical compounds such as ethanol in water. Up to 13 volatile compounds was identified in the condensate using GC/MS and was quantified using a FID detector. Results from analysis of alcohols, esters and aldehydes in condensate (0°C) and trap (-40°C) collected from the stripping of Standard 1100 fermentation are shown in Figure 5–46 to Figure 5–52. Changes in concentrations of those compounds followed approximately the same pattern in the condensate and in the trap. Changes in condensate and trap concentration of isoamyl alcohol, isobutanol, propanol, ethyl acetate, acetaldehyde, 2-phenylethyl acetate and isoamyl acetate during the fermentation followed similar trend as for the changes in ethanol concentration (Figure 5–7). As demonstrated in section 5.1.9.1, changes in condensate concentrations of isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde followed closely the changes in concentration of ethanol, with a decrease in concentration from day 3 towards a peak in concentration between day 10 and day 12. Furfuryl alcohol and phenyl ethanol did not follow the same pattern as their respective concentration gradually increased from day 4 to the end of the stripping period without any peak of concentration. The results for ethyl caprylate, n-butanol, hexanol and isobutyl acetate were not as clear as for the other compounds, possibly due to their very low concentration, which was probably near the limit of detection of the analytical method.

The condensate and trap fractions constitute a natural source of beer volatile compounds. The total concentration of the different compounds is shown in Table 5-28. The ratio of trap to condensate concentration in each volatile compound was calculated and listed in Figure 5–29. For example, isoamyl alcohol and ethyl acetate were between 5 to 7 and between 49 to 101 times more concentrated in the trap than in the condensate respectively. This confirmed that ethyl acetate was more volatile than isoamyl alcohol, as discussed in Chapter 4. Contrary to these compounds,

furfuryl alcohol, which is less volatile, was between 3 and 5 times more concentrated in the condensate than in the trap. As expected, the trap (-40°C) was more concentrated in the most volatile compounds, and the condensate (0°C) in the least volatile compounds. The ratios were compared to their volatility as estimated in Chapter 4. The ascending order of the trap/condensate ratio followed quite closely the ascending order of volatility, except for hexanol, whose quantification in condensate and trap was impaired by the limit of detection of the analytical method.

Table 5-28: Volatile compound concentrations in total fraction of condensate and trap.

Volatile compound	Condensate concentration ($mg.L^{-1}$)				Trap concentration ($mg.L^{-1}$)	
	Standard 1100	Non-agitated 1100	Non-aerated 1100	Standard 1080	Standard 1100	Non-agitated 1100
Acetaldehyde	4.4	3.8	3.9	4.9	39.5	40.4
Ethyl acetate	1.3	1.7	2.4	3.8	99.2	303.3
Methanol	0.0	0.0	N/A	N/A	5.1	6.1
Isobutyl acetate	0.0	0.0	N/A	N/A	0.2	4.6
Propanol	62.0	45.0	60.2	33.5	264.8	184.0
Isobutanol	53.7	112.4	187.7	73.1	388.5	795.1
Isoamyl acetate	0.0	0.2	N/A	N/A	39.2	53.3
n-butanol	0.9	0.4	N/A	N/A	4.5	0.0
Isoamyl alcohol	394	430.3	616.0	245.5	2550	2619.3
Ethyl caproate	0.0	0.0	N/A	N/A	0.0	0.0
1-hexanol	0.4	0.0	N/A	N/A	0.1	0.0
Ethyl caprylate	0.6	0.0	N/A	N/A	192.9	61.8
Ethyl caprate	0.0	0.0	N/A	N/A	0.0	0.0
Furfuryl alcohol	3.4	3.1	N/A	N/A	1.3	0.7
2-phenylethyl acetate	1.9	0.8	N/A	N/A	6.4	0.0
2-phenyl ethanol	25.7	17.3	N/A	N/A	5.3	4.1
Condensate/trap volume (ml)	753	738	952	861	516	333

Table 5-29: Ratio of volatile compound concentrations between trap and condensate in Standard 1100 fermentation compared to the K value calculated in Chapter 4, listed in the ascending order of K values.

Volatile compound	Trap/condensate concentration ratio	K value Unifac 5%v/v
2-phenyl ethanol	0.2	0.020
Furfuryl alcohol	0.2-0.6	0.034
2-phenylethyl acetate	3-4	0.077
n-butanol	3-7	0.139
Hexanol	0-0.2	0.160
Propanol	4-5	0.172
Isoamyl alcohol	5-7	0.227
Isobutanol	6-8	0.313
Acetone	6-12	1.419
Acetaldehyde	6-9	3.634
Ethyl acetate	49-101	4.717
Isobutyl acetate	N/A	6.751
Ethyl caprylate	35-416	144.226

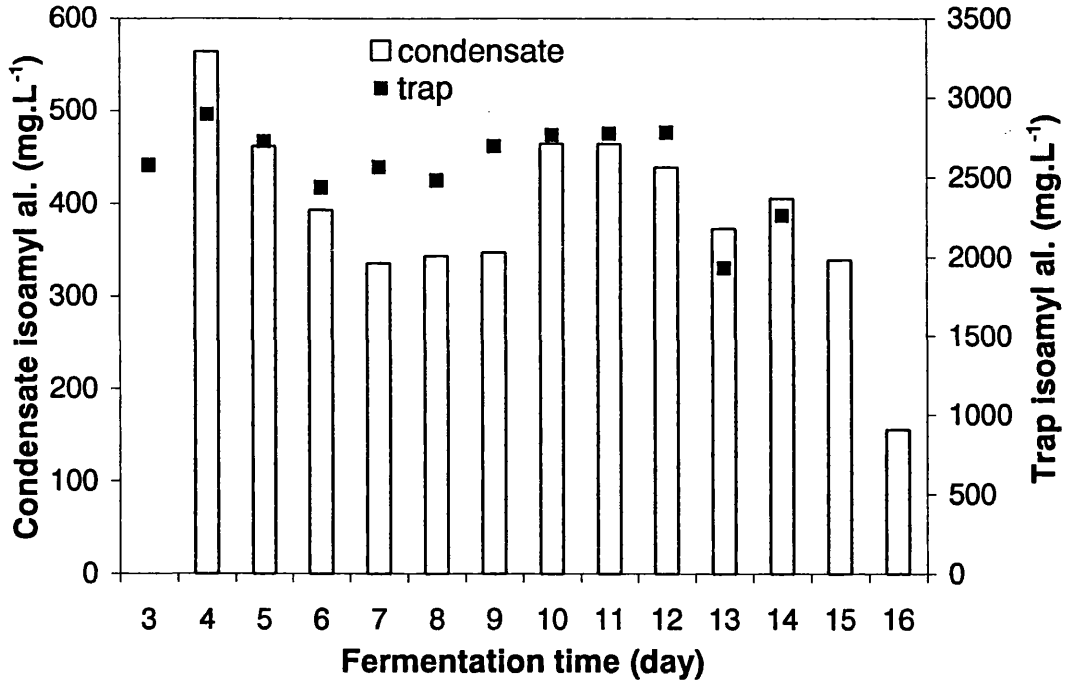

Figure 5–46: Changes in isoamyl alcohol concentration in condensate (0 ℃) and trap (-40 ℃) collected during CO_2 stripping of Standard 1100 fermentation.

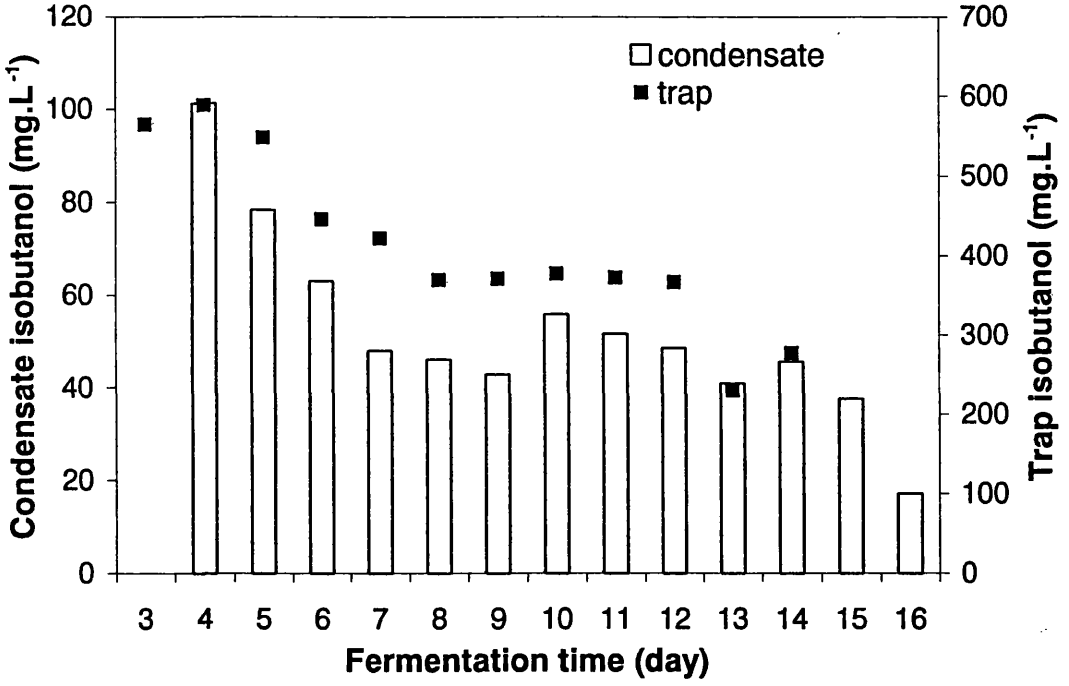

Figure 5–47: Changes in isobutanol concentration in condensate (0 ℃) and trap (-40 ℃) collected during CO_2 stripping of Standard 1100 fermentation.

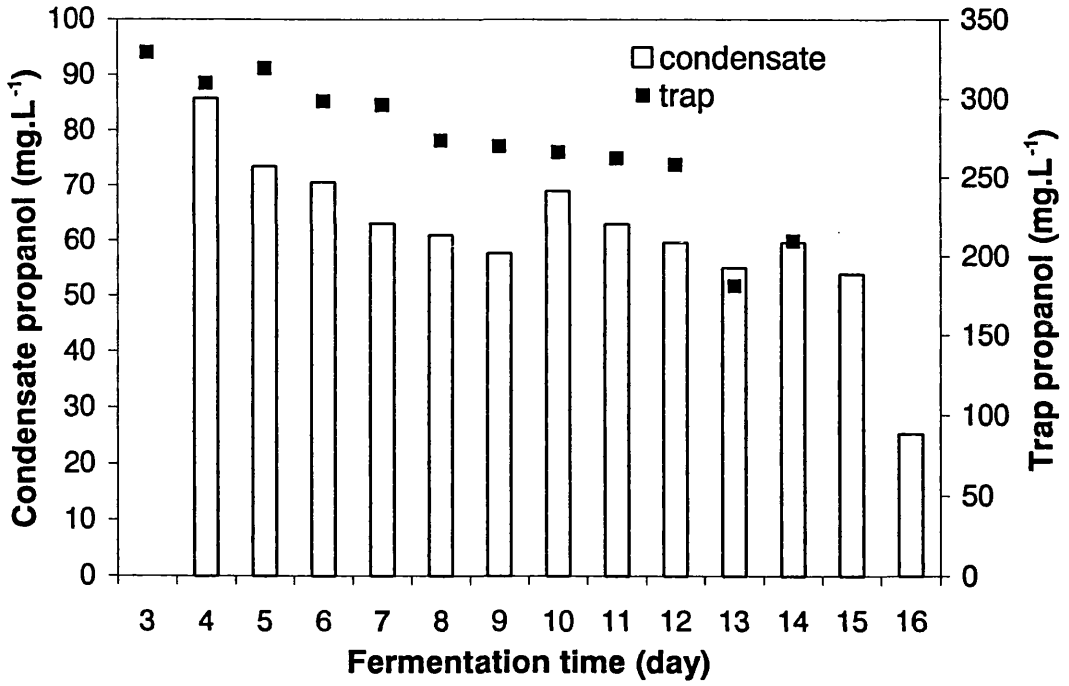

Figure 5–48: Changes in propanol concentration in condensate (0 ℃) and trap (-40 ℃) collected during CO_2 stripping of Standard 1100 fermentation.

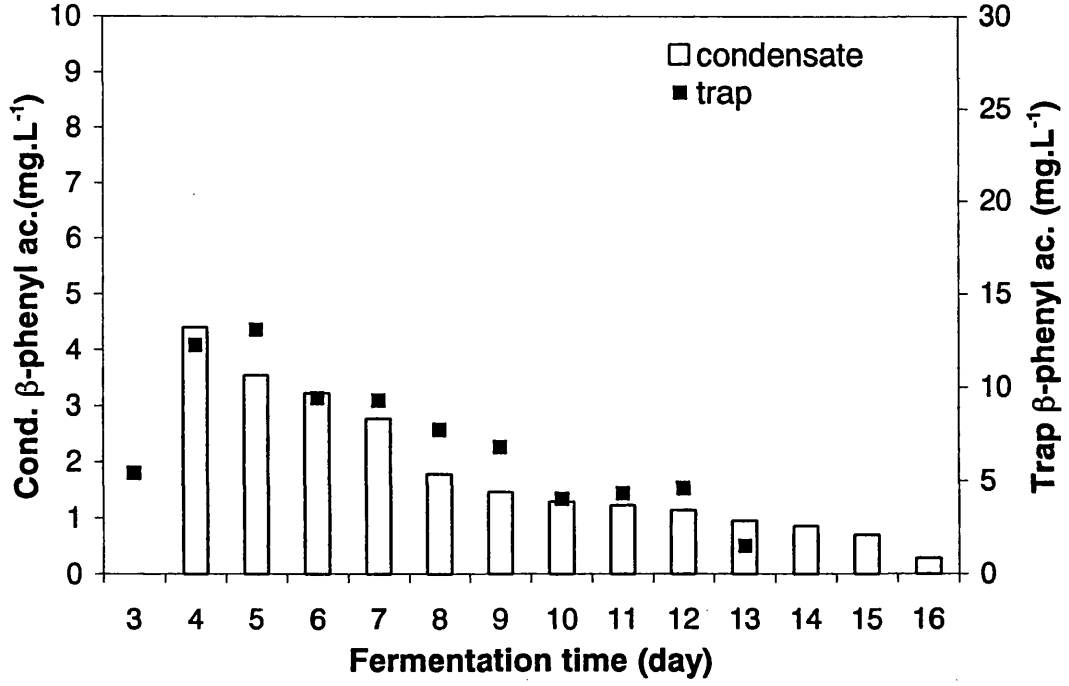

Figure 5–49 : Changes in 2-phenylethyl acet. concentration in condensate (0 ℃) and trap (-40 ℃) collected during CO_2 stripping of Standard 1100 fermentation.

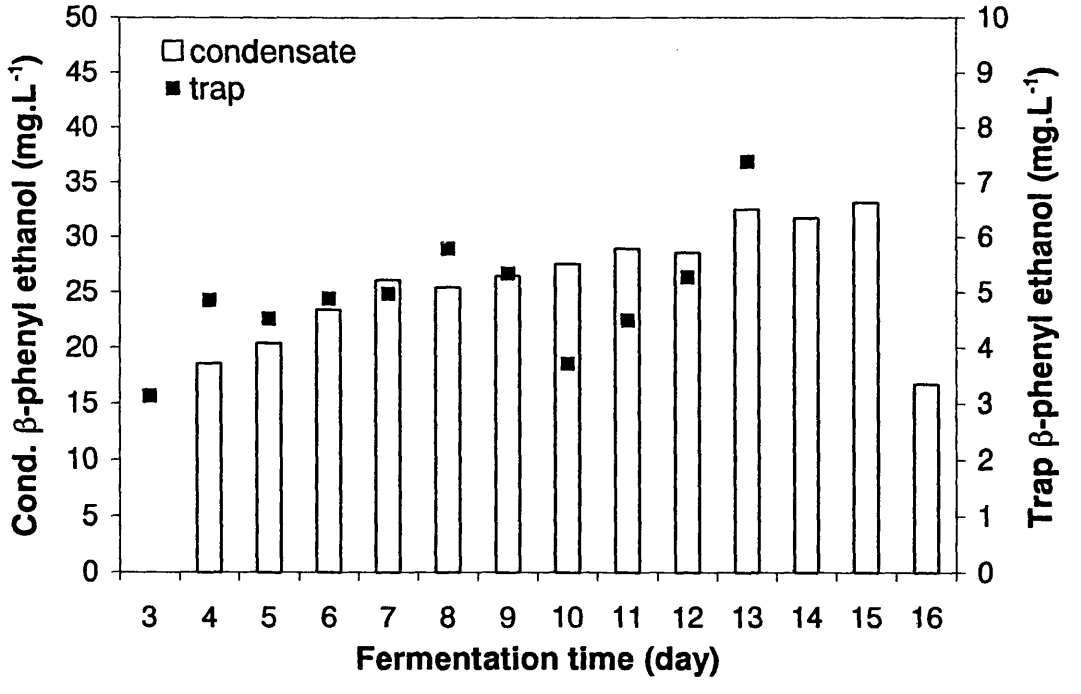

Figure 5–50 : Changes in phenyl ethanol concentration in condensate (0 °C) and trap (-40 °C) collected during CO_2 stripping of Standard 1100 fermentation.

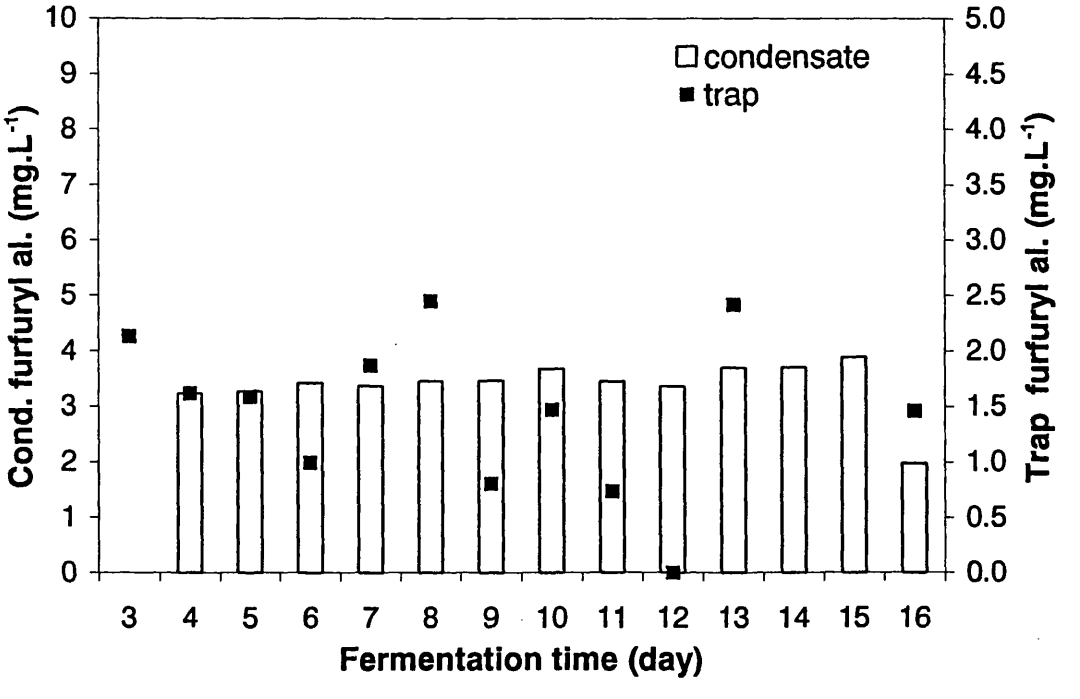

Figure 5–51 : Changes in furfuryl alcohol concentration in condensate (0 °C) and trap (-40 °C) collected during CO_2 stripping of Standard 1100 fermentation.

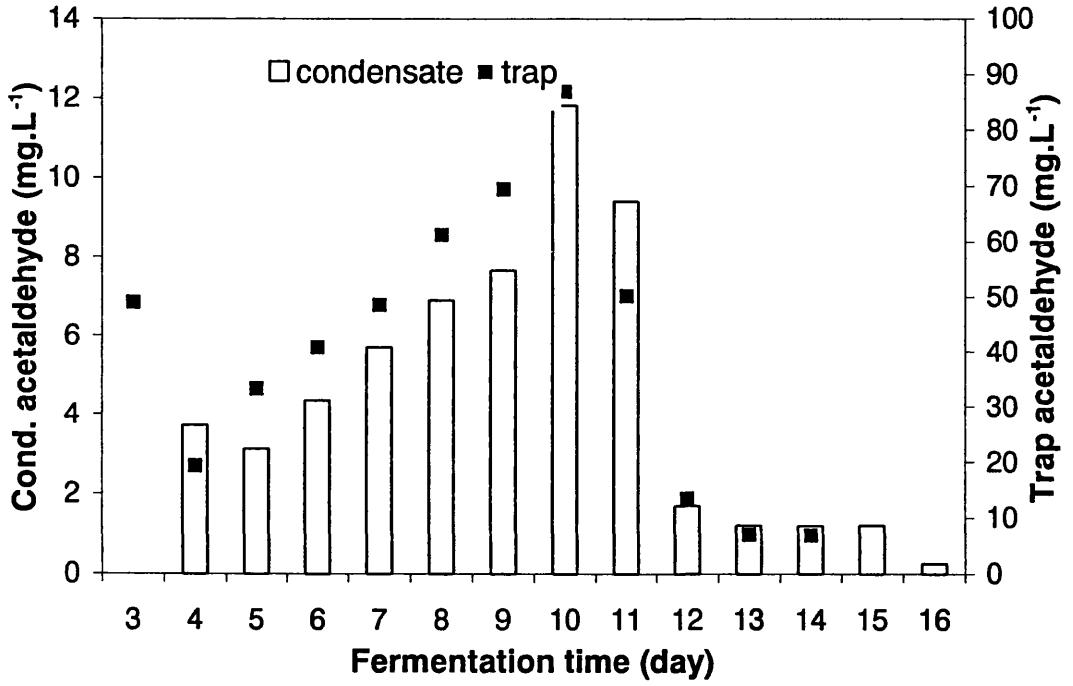

Figure 5–52: Changes in acetaldehyde concentration in condensate (0 °C) and trap (-40 °C) collected during CO_2 stripping of Standard 1100 fermentation.

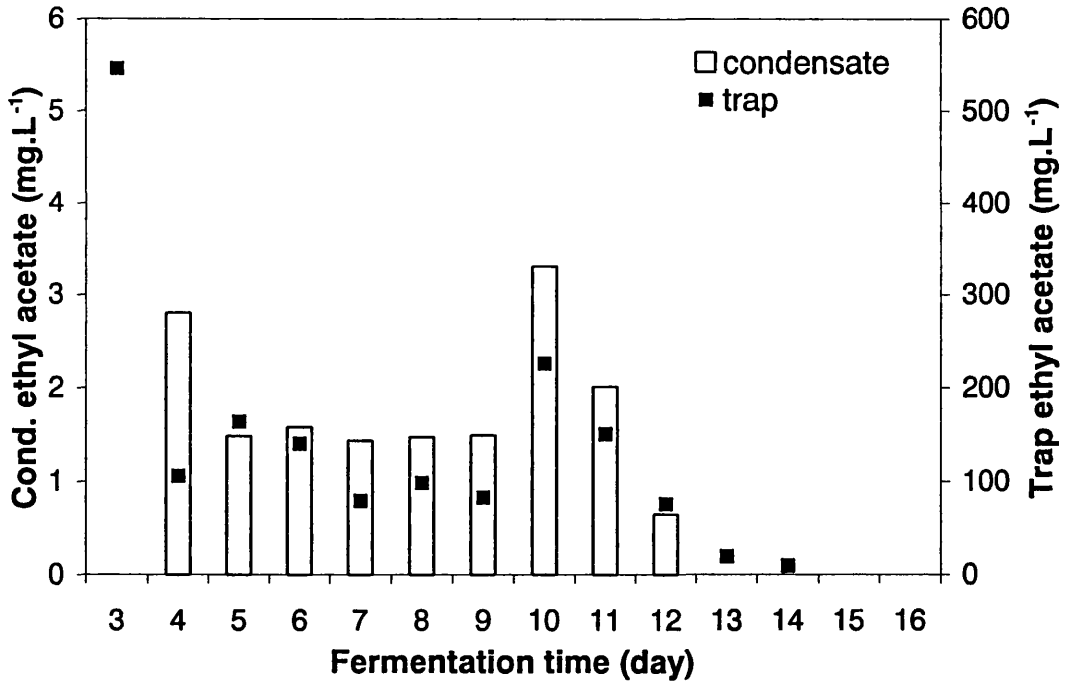

Figure 5–53: Changes in ethyl acetate concentration in condensate (0 °C) and trap (-40 °C) collected during CO_2 stripping of Standard 1100 fermentation.

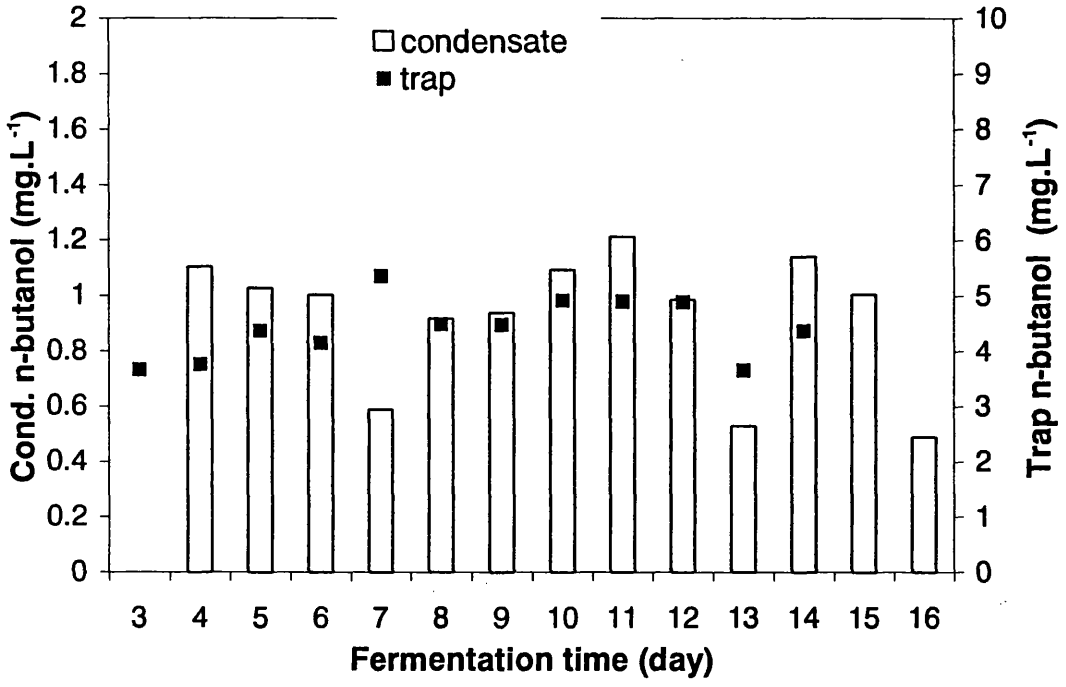

Figure 5–54: Changes in n-butanol concentrations in condensate (0 ℃) and trap (-40 ℃) collected during CO_2 stripping of Standard 1100 fermentation.

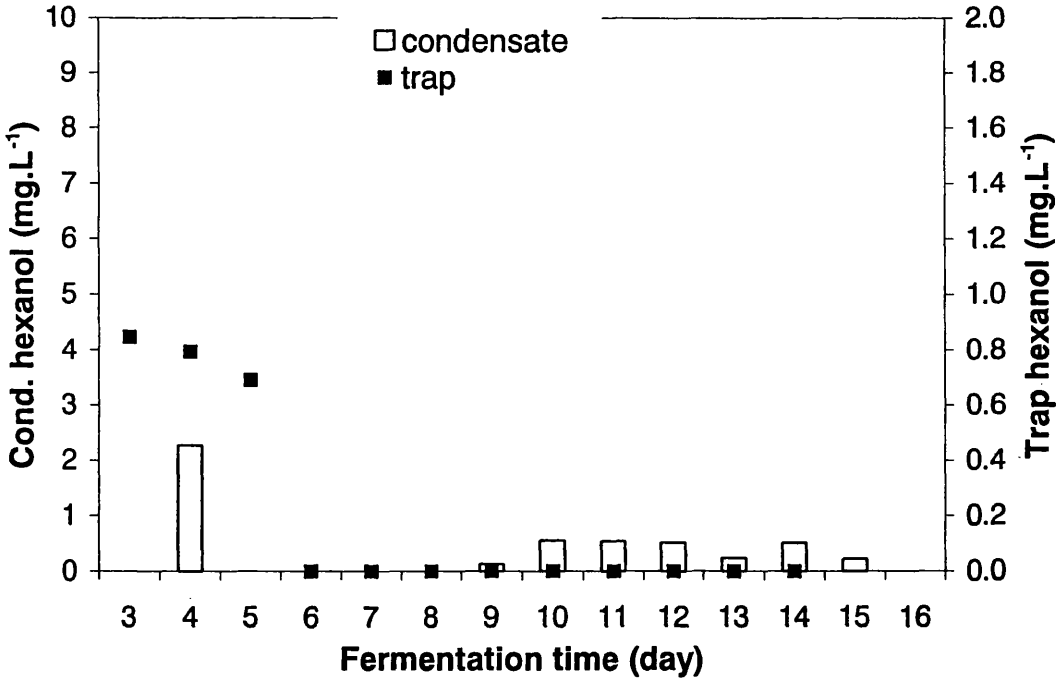

Figure 5–55: Changes in hexanol concentration in condensate (0 ℃) and trap (-40 ℃) collected during CO_2 stripping of Standard 1100 fermentation.

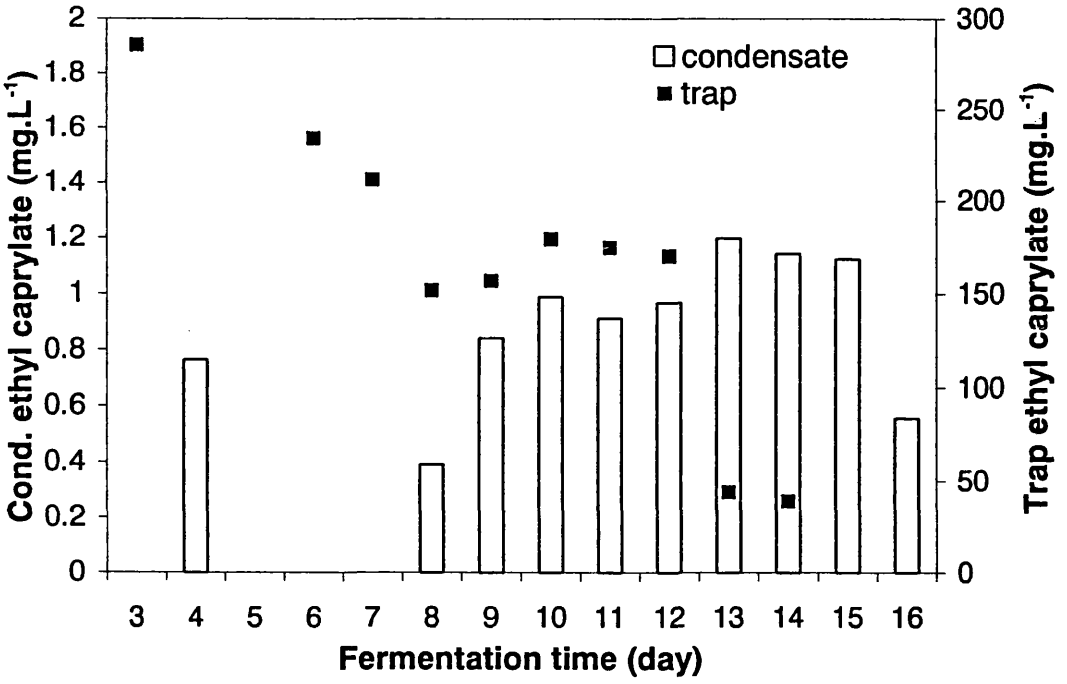

Figure 5–56: Changes in ethyl caprylate concentration in condensate (0 °C) and trap (-40 °C) collected during CO_2 stripping of Standard 1100 fermentation.

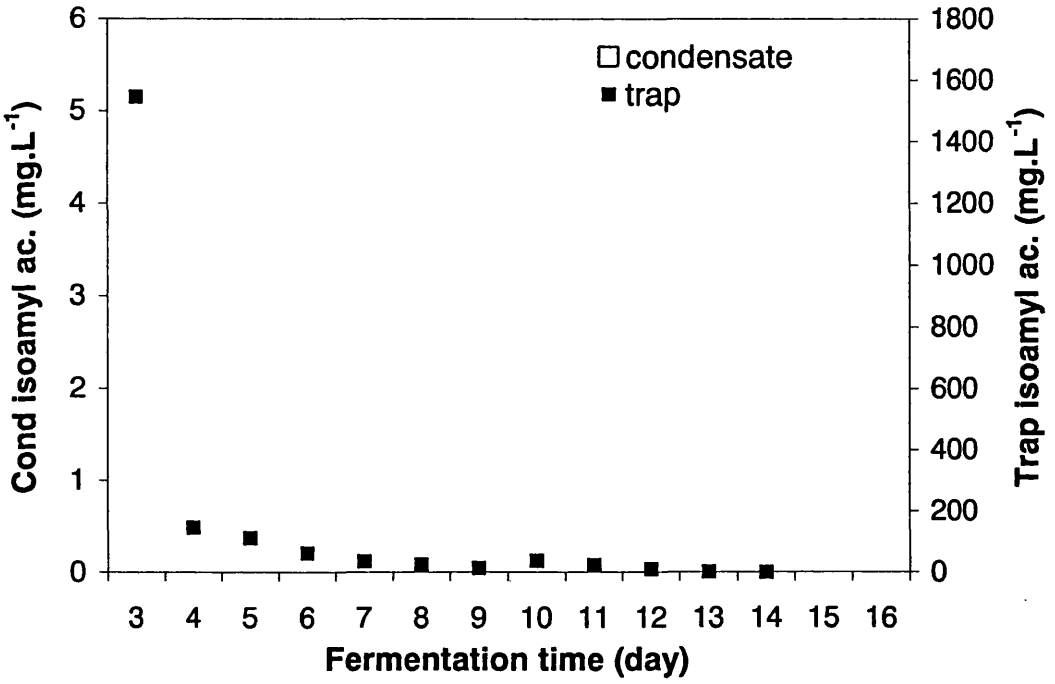

Figure 5–57 : Changes in isoamyl acetate concentration in condensate (0 °C) and trap (-40 °C) collected during CO_2 stripping of Standard 1100 fermentation.

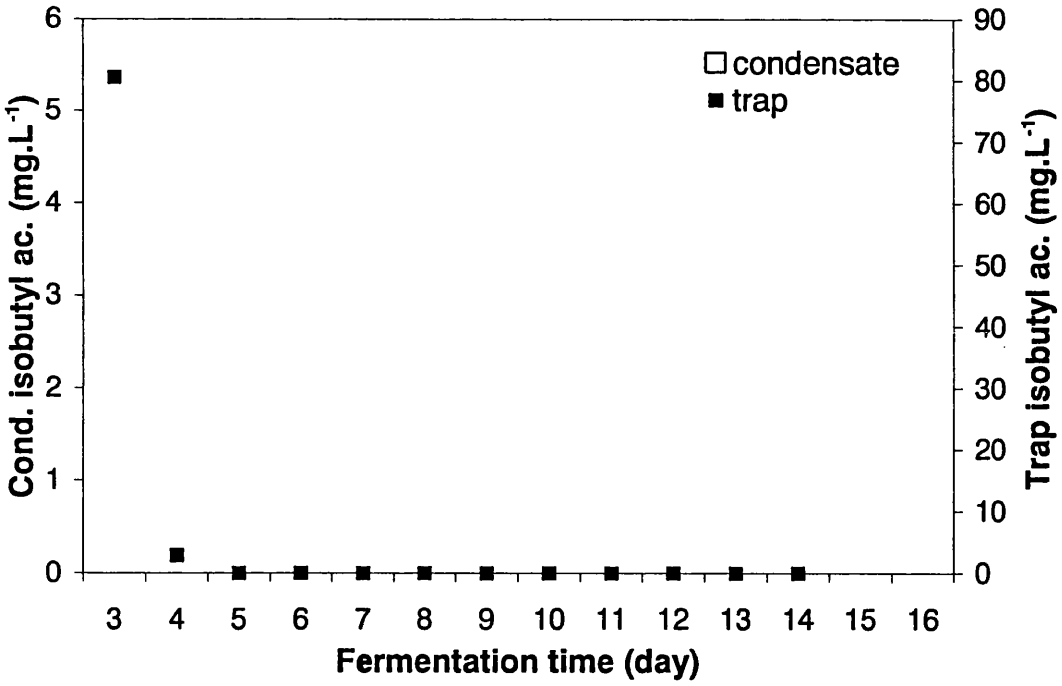

Figure 5–58: Changes in isobutyl acetate concentration in condensate (0 ℃) and trap (-40 ℃) collected during CO_2 stripping of Standard 1100 fermentation.

5.1.12 Effect of stripping on the beer flavour profile

Quantification of volatile compounds using direct injection of the beer samples onto a packed column was limited to the main beer volatile compounds; isoamyl alcohol, isobutanol, propanol, acetaldehyde and ethyl acetate (section 5.1.9). The limitations of the chromatographic method did not allow the analysis of the minor, but flavour-active, volatile compounds from the fermentation medium. Therefore, development of another gas chromatographic method using a sampling technique, which would enable the concentration of the minor compounds prior to injection, was carried out, as described in Chapter 3. A dynamic headspace technique, where the volatile compounds were concentrated using purge and trap followed by thermal desorption onto a capillary column was used. The different compounds were identified by mass spectrometry. Due to the lack of technical, economic and time resources, an extensive development of the method was not possible to include within the scope of this project. Therefore, only preliminary results using that method are presented in this

section. This provides, however, an illustration of the potential of the dynamic headspace technique for the analysis of flavour compounds from fermentation medium.

Compounds usually found in typical beer (a commercial Carlsberg lager was analysed using the same headspace technique for comparison purposes) were identified in the beer medium of the stripped and control fermentations. Although a limited number of compounds were identified (as shown in Table 5-30), the flavour profiles of the control and stripped fermentations provided a means of differentiating between them. The higher alcohols were not well identified, as only isobutanol and furfuryl alcohol were present. However, short chain acids (formic acid, acetic acid), fatty acids (pentanoic acid, caproic acid, caprylic acid) and glycerol, another by-product of yeast metabolism were identified. To provide a complete spectrum of volatile compounds, parameters such as the purge gas flow rate, the purge time, the purge temperature and the adsorbent material should be optimised. A difference in the retention time of the compounds eluted in the first 25 minutes was found between control and stripped beers. As the method was under development, a few parameters on the Automated Thermal Desorption unit were not controlled accurately and resulted in this discrepancy.

Figure 5–59 shows the chromatograms obtained for a control beer fermentation, a stripped beer fermentation and a commercial Carlsberg lager. Ethanol was allowed to elute from the column before switching on the mass spectrometer, as the filament is very sensitive and a large quantity of solvent would damage it. The runs were therefore initiated after 10 minutes of the injection (desorption). However for chromatogram A (control beer fermentation), the MS was switched on before the complete elution of ethanol, in order to visualise if any important compounds were present within that period. Two peaks were observed between 5 and 10 minutes, but have not been well identified. Peaks 9 and 12, corresponding to pentanoic acid and hexanoic acid respectively, were present in the control fermentation but not in the stripped fermentation. Peak 17, corresponding to ethyl pentanoate was only found in the stripped fermentation. The ratios of the area of peak 10 to peak 5 increased in the stripped fermentations compared to the control fermentations, which suggests that stripping changes the flavour balance by creating a disproportionate concentration of

pentanal over furfuryl alcohol. The ratios of the area of peak 10 to peak 1, 3 and 4 decreased in the stripped fermentations compared to the control fermentations, which suggests either a lower production of isobutanol, acetic acid and formic acid, or a higher stripping rate of those compounds. In summary, the results, although limited, show that stripping resulted in total or partial removal of some flavour-active compounds and probably in the production of other compounds. To enable a better comparison between stripped and control fermentations, optimisation of the analytical technique is required. Quantification (using calibration solutions and the use of an internal standard) of the various compounds in both the stripped medium and condensate should also be investigated as it would enable the calculation of the net production of these compounds (providing that the condensation unit has been optimised).

Table 5-30: Beer volatile compounds identified in control and stripped fermentations and in a commercial Carslberg lager using the ATD-GC/MS technique.

Peak N°	Component name	Retention time (min)		
		Control	Stripped	Carlsberg
1	Isobutanol	19.1	15.5	19.0
2	Methanol	19.8	16.7	19.6
3	Acetic acid	20.6	18.7	20.4
4	Formic acid	21.8	20.9	21.4
5	Furfuryl alcohol	24.5	23.7	24.4
6	2(5H)furanone	27.3	27.0	27.1
7	Ethyl pentanoate		27.8	
8	Maltol	32.9	33.1	32.54
9	Pentanoic acid	34.5		
10	Pentanal	36.6	36.7	36.4
11	Hexanoic acid (caproic)	38.9	38.8	38.4
12	Octanoic (caprylic)acid	39.7		39.1
13	Glycerol	43.2	43.7	42.9

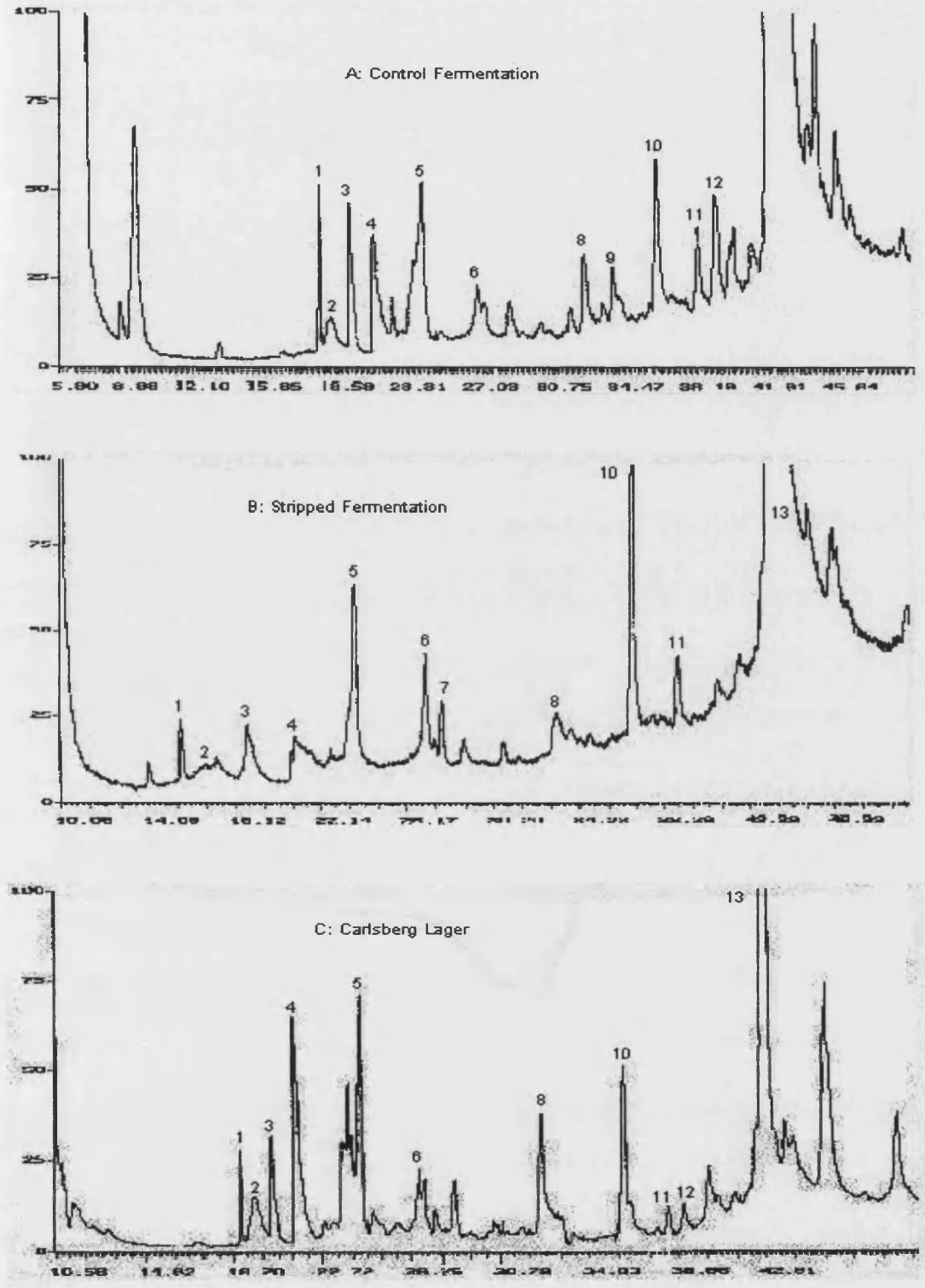

Figure 5–59: GC/MS chromatograms (TIC) for a control beer fermentation (A), a stripped beer fermentation (B) and a commercial Carlsberg lager (C), analysed using the ATD-CG/MS technique.

5.1.13 Feasibility studies into the use of pervaporation for concentration/fractionation of beer condensate volatile compounds.

Beer condensate, recovered after CO_2 stripping, constitutes a natural source of beer flavour compounds. If the stripped fermentations (as produced in the present study) were to be used as low-alcohol beverages, their flavour profile should be adjusted, as most of the flavour compounds have been reduced by different ratio with stripping. Ethanol and water should also be separated from the condensate before adding the flavour concentrate back into the fermenter. On the other hand, if the stripped fermentations were to be used to increase the efficiency of the brewing process, ethanol should be wholly added back into the fermenter. However, as stripping produce a disproportionate amount of some compounds, the condensate should be fractionated in order to add the desirable flavour compounds back into the fermenter. In either case, a concentration and possibly a fractionation of the beer condensate is required. Pervaporation was investigated as a method of concentrating the condensate further. As a preliminary study, the pervaporation experiments were carried out with synthetic mixtures containing ethanol, isoamyl alcohol, isobutanol and propanol. The four compounds were the major components of condensates collected from CO_2 stripped beer fermentations. The performance of the pervaporation process was assessed by the enrichment factor of the volatile compound, which is defined by:

$$\beta = w^{perm}/w^{feed}$$

where, w^{perm} and w^{feed} are mass fractions of the volatile compound in the permeate and feed respectively.

5.1.13.1 Effect of the membrane on the enrichment factors.

The performance of a standard PDMS (Polydimethyl siloxane) membrane was compared with a modified PDMS membrane (supplied by GKSS). The pervaporation experiments were carried out with synthetic mixtures containing 0.5 $g.L^{-1}$ of ethanol, propanol, isobutanol and isoamyl alcohol. Figure 5–60 shows the effect of the membrane on the enrichment factor of the different compounds. With the standard PDMS membrane, concentrations of ethanol, propanol, isobutanol and isoamyl alcohol were respectively 6, 15, 34 and 60 times higher in the permeate than in the

feed. The enrichment factors increased with carbon chain length of the volatile compound ($\beta_{\text{isoamyl alcohol}} > \beta_{\text{isobutanol}} > \beta_{\text{propanol}} > \beta_{\text{ethanol}}$).

With the modified PDMS membrane, the enrichment of the different volatile compounds was higher than with the standard PDMS membrane. The enrichment factors of ethanol, propanol, isobutanol and isoamyl alcohol increased by 35, 39, 46 and 50% respectively. The higher enrichment was attributed to the higher hydrophobicity of the membrane, due to a high proportion of long chain alkyl groups (detailed information on the membrane was not provided by the supplier). It was also observed that higher the hydrophobicity (increased carbon chain length) of the volatile compound, higher the percentage increase of the enrichment factor. The GKSS membrane was relatively thin and gave fluxes of 120, 0.6 and 9.7 $g.m^{-2}.h^{-1}$ for water, ethanol and the organic compounds respectively. The fluxes with the PDMS membrane were 51, 0.2 and 3 $g.m^{-2}.h^{-1}$ for water, ethanol and the organic compounds respectively.

5.1.13.2 Effect of ethanol concentration on the enrichment factors.

The results for the pervaporation of the synthetic mixture containing 0.5 $g.L^{-1}$ of each volatile compound (equi-concentration solution) was compared with the results found with an artificial beer condensate (synthetic mixtures containing volatile compounds concentrations found in real beer condensates) containing 100 $g.L^{-1}$ (12.7 % v/v) ethanol, using a PDMS membrane.

The relatively high concentration of ethanol in the artificial condensate affected the permeation of the other volatile components, decreasing their enrichment factor (Figure 5–61). In a feed solution containing 100 $g.L^{-1}$ethanol, the enrichment factor of propanol, isobutanol and isoamyl alcohol decreased by 8, 74 and 77% respectively compared with a feed solution containing 0.5 $g.L^{-1}$ ethanol. The presence of ethanol adversely affected the activity coefficients of isoamyl alcohol and isobutanol, thus reducing the activity difference (and hence the component fluxes) across the membrane. The higher the hydrophobicity of the volatile component the greater the effect of ethanol. The fluxes were 54, 22, and 1.4 $g.m^{-2}.h^{-1}$ for water, ethanol and the other organic compounds respectively.

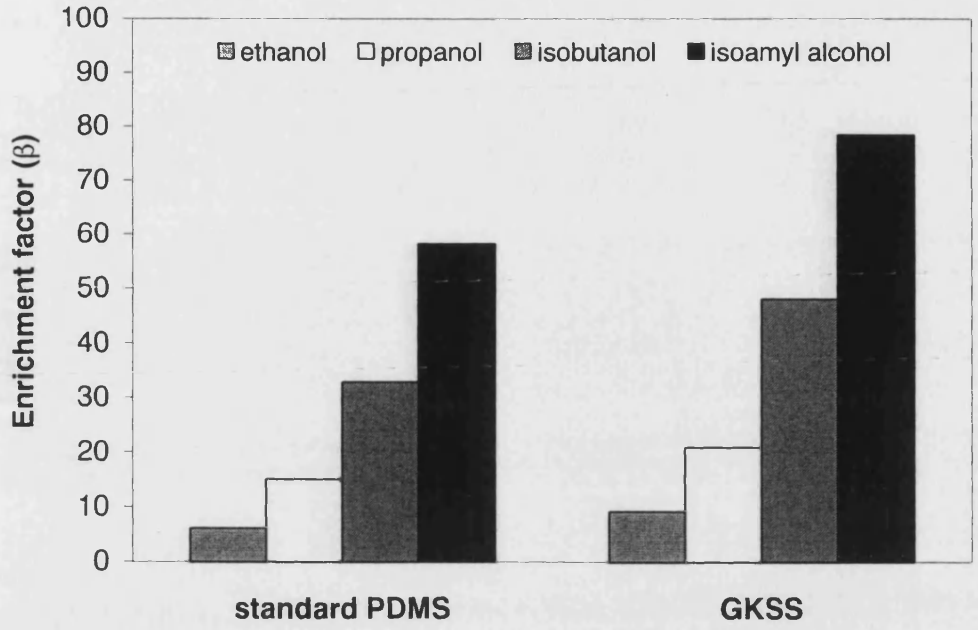

Figure 5–60: Effect of the membrane on the enrichment factor of beer condensate volatile compounds (feed containing 0.5 g.L^{-1} of each volatile compound, maintained at 40°C).

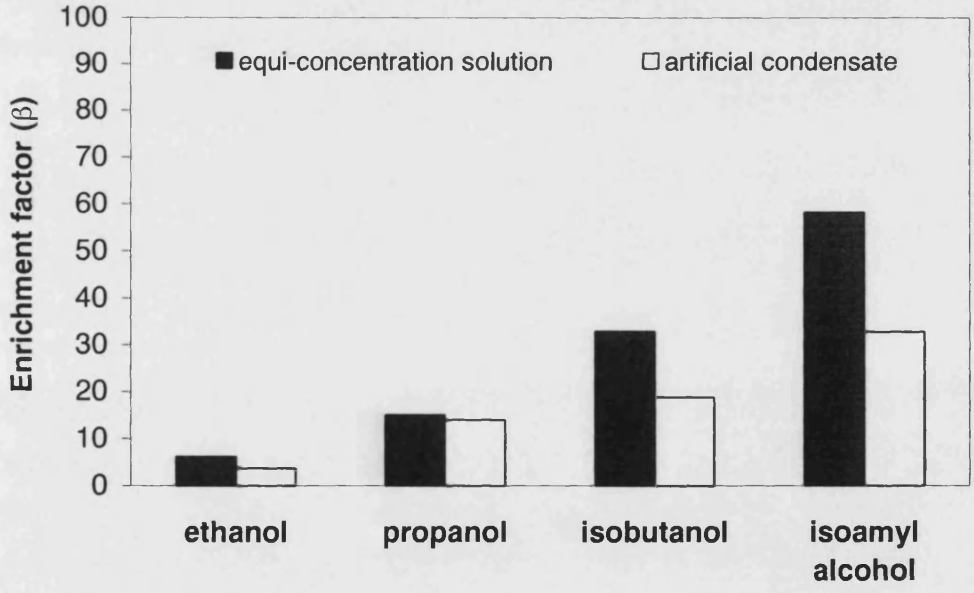

Figure 5–61: Effect of ethanol on the enrichment factor of beer condensate volatile compounds. The equi-concentration solution and the artificial beer condensate contain 0.5 g.L^{-1} and 100 g.L^{-1} ethanol respectively (PDMS membrane, 40°C).

5.1.14 Summary of results

The results for the CO_2 stripping of high original gravity beer fermentation using exogenous CO_2 (no recirculation of the gas) can be summarised as follows:

- Medium stripped ethanol content was decreased by approximately 50% on average by continuously stripping the fermentation for over 14 days. Medium stripped ethanol reached a maximum level in the medium after 10-12 days of fermentation, while control ethanol reached a plateau. After the maximum, further stripping forced medium stripped ethanol to decrease.

- Continuous CO_2 stripping resulted in a higher metabolism of the sugars present in the medium. The uptake of glucose, fructose and sucrose was the same in both the control and the stripped medium. However, a higher uptake of maltose was observed in all stripped fermentations.

- The higher sugar uptake was confirmed by a higher rate of fermentation in the stripped fermentation than in the control fermentation, as shown by the changes in corrected specific gravity (correction with both the trap and condensate).

- The lack of aeration did not affect ethanol production or the sugar uptake of the stripped fermentation compared to the standard stripped fermentation. However, the lack of agitation, by recirculation of the fermenting medium, reduced ethanol production and sugar uptake.

- Stripping did not adversely affect the medium pH profile. Only a slight, but consistent, decrease of pH by 0.1-0.2 units was observed in the stripped medium compared to the control.

- Stripping affected cell physiology and morphology. Cell growth, cell viability and budding were higher in the stripped fermentations than in the control fermentations. Cell size distribution shifted towards a greater number of larger cells, and cell biomass was also higher in the stripped fermentations compared to the control fermentations.

- Despite a higher sugar consumption in the stripped medium and a faster rate of fermentation, the apparent net ethanol production in the stripped medium was lower than in the control medium. However, due to the inefficiency of the condensation unit (as observed in Chapter 4), ethanol was not totally recovered. Correction for ethanol loss, using percentage recovery estimated for synthetic mixtures (Chapter 4), resulted in a 29% over estimation when compared to the maximum theoretical ethanol that the fermentation could produced.

- The concentration in the other beer volatile compounds such as isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde were also decreased by continuous stripping of the medium. Net production of these compounds was also lower in the stripped medium than the control. As for ethanol, the inefficiency of the condensation unit resulted in a loss of the volatile flavour compounds. A correction using percentage recoveries resulted in a higher production of these compounds in the stripped medium compared to the control medium.

- Changes in the condensate and trap beer volatile compounds showed different patterns of production for the different volatile compounds. Isoamyl alcohol, isobutanol, propanol, ethyl acetate, acetaldehyde, 2-phenylethyl acetate and isoamyl acetate production concentrations followed closely condensate ethanol concentration. Their concentration in the condensate peaked up around day 10-12 before being decreased by further stripping. However, furfuryl alcohol and 2-phenyl ethanol followed a different pattern, as their concentration in the condensate continually increased until the end of the fermentation.

- Pervaporation provided a means of concentrating the volatile compounds from a beer condensate produced by CO_2 stripping. Pervaporation performance was sensitive to the hydrophobicity of the volatile compounds, the hydrophobicity of the pervaporative membrane and the level of ethanol in the condensate.

5.2 DISCUSSION

5.2.1 Introduction

Fermentation results obtained with CO_2 stripping are given in section 5.1 of this chapter. The present work is a continuation of previous work carried out in this laboratory by Huxtable [1993], who exploited the use of in-situ CO_2 stripping for the production of low-ethanol cider. Cider fermentations were carried out in 35L tower fermenters and the CO_2 produced by the fermentation was recirculated through the medium. It was found that medium ethanol levels could be reduced to a minimum of 2.9% with an original gravity of 1068 and a temperature of 22°C. Despite a higher sugar uptake, net ethanol production in the stripped cider medium was lower than in the control fermentation. With higher gravity fermentation (OG 1090), the results were different as ethanol production was slightly higher in the stripped medium than in the control. Due to the relatively higher pressure ($5x10^4$ Pa), measured dissolved CO_2 in the stripped medium was higher than in the control at 0.33% compared to 0.10%. The results found by Huxtable were therefore attributed to a combination of removal of ethanol inhibition by lowering medium ethanol and of an increase in CO_2 inhibition. However, due to the complexity of the fermentation system, the different results obtained between normal gravity (OG 1068) and high gravity (OG 1090) were not fully understood.

The present fermentation system has been simplified by carrying out the various fermentations in smaller and more flexible fermenters (10L), in duplicate and also at atmospheric pressure (foaming was controlled by the addition of an antifoam). As the experiments were carried out at atmospheric pressure, the dissolved CO_2 concentration was a function of ethanol concentration only. The effects due to stripping were expected to be dominated by the removal of ethanol inhibition and not by CO_2 inhibition. The following sections present a discussion on the various results obtained during stripping experiments of high gravity beer fermentations. The results obtained with stripped fermentations are compared with control fermentations where no stripping was used.

5.2.2 Effect of CO_2 stripping on medium ethanol level

5.2.2.1 Standard fermentations

The main objective of CO_2 stripping was to reduce medium ethanol content below the yeast inhibitory level, in order to enable complete fermentation of high gravity media. Both control fermentations with an original gravity of 1080 and 1100, which were carried out under standard conditions (initial aeration of the beer wort and continuous mechanical agitation of the medium), produced a maximum of 67-68 $g.L^{-1}$ final volume (8.5-8.7 % v/v) of ethanol within 11-12 days of fermentation. Fermentation stopped when medium ethanol reached the ethanol tolerance level (no more ethanol was produced after day 15). The concentration of 8.5-8.7 % v/v was therefore attributed to the ethanol tolerance level of the ale yeast used in this work under the present conditions of fermentation. With stripping, the ethanol level in all stripped fermentations was always kept under that of the control. A maximum of 51 $g.L^{-1}$ (6.5% v/v) and 45 $g.L^{-1}$ (5.7 %v/v) of ethanol was reached in the standard fermentation with OG 1100 and OG 1080 respectively compared to 66 $g.L^{-1}$ in the control. Not only did CO_2 stripping maintain ethanol under the toxic level, but it enabled the production of depleted-ethanol beer. Final ethanol concentrations (day 14, corrected for volume loss) were 34 $g.L^{-1}$ (4.3 %v/v) and 37 $g.L^{-1}$ (4.7 % v/v) in Standard 1080 and Standard 1100 fermentations respectively, which were approximately half the maximum concentration reached by the respective control media. Dilution of the stripped media, as it is usually carried out in breweries using high gravity wort, would reduce further the ethanol content (i.e. a 50% dilution would yield approximately 2-2.5 % v/v ethanol) and produce a low-alcohol beer.

5.2.2.2 Non-agitated fermentation

Ethanol production (66 $g.L^{-1}$) in the control standard fermentation with OG 1100 was improved by 27% compared with a non-agitated fermentation (52 $g.L^{-1}$). When stripping was applied, ethanol was also kept under a lower ethanol level at 40 $g.L^{-1}$ in the non-agitated medium compared to 51 $g.L^{-1}$ in the standard medium. Final ethanol concentration on day 14 (corrected for volume loss) was also lower at 33 $g.L^{-1}$ compared to the standard at 37 $g.L^{-1}$. The lower ethanol concentrations (final and maximum) in the non-agitated fermentations compared to the standard fermentations

was attributed to a lower ethanol production rate, due to the lack of mechanical agitation (through recirculation of the medium via a peristaltic pump).

5.2.2.3 Non-aerated fermentation

The non-aerated control fermentation with OG 1100 was characterised by a slower ethanol production rate. By day 14 of the fermentation, only 57 $g.L^{-1}$ initial volume of ethanol was produced compared to 66 $g.L^{-1}$ in the standard control fermentation with OG 1100. The fermentation took another two days to reach the same medium ethanol concentration as found in the standard fermentation (67-68 $g.L^{-1}$ final volume). However, non-aerated stripped medium behaved similarly to the standard stripped medium. Final ethanol concentration on day 14 (corrected for volume loss) was 40 $g.L^{-1}$comparable to 37 $g.L^{-1}$ in the standard fermentation. The maximum ethanol concentration reached in the non-aerated fermentation was also the same at 51 $g.L^{-1}$. CO_2 stripping, as well as having an agitation effect, seemed to have an aeration effect, and media, which have not been aerated before pitching, fermented similarly to aerated media under stripping conditions.

5.2.2.4 Continuous stripping

Changes in continuously stripped ethanol concentrations in all but the non-agitated fermentations followed a similar pattern, with a gradual increase of the concentration until day 10 to day 13 (varying from one fermentation to another). Ethanol concentration increased gradually in the stripped beer as if the ethanol production rate was higher than the stripping rate. Days 10-13 was also the time where ethanol concentration in the control beer reached a plateau, and where no more ethanol was produced, due to ethanol inhibition. From day 10-13, the stripped ethanol concentration decreased to the end as if the stripping rate was higher than the production rate. This result was surprising as ethanol concentration in the stripped beer was kept below the ethanol tolerance level of the yeast species, and was expected to allow for further fermentation. This ethanol reduction was probably due to production of other toxic by-products, or to insufficiency of essential nutrients or available oxygen.

5.2.2.5 Intermittent or periodic stripping

In the periodically stripped fermentation with OG 1080, periods of stripping (between 2 or 3 days) where medium ethanol was kept almost constant was followed by periods of non-stripping where ethanol increased at a similar rate to the control fermentation. This result shows that the short exposures to CO_2 did not affect the fermentative activity of the yeast species, which continued to ferment the medium at the same rate as in the control fermentation. In other words, the yeast cells, which had a history of increased exposure to CO_2 (during the stripping periods), behaved similarly to the control cells when stripping was removed. During the last period of stripping (day 12 to day 17), ethanol concentration in the beer medium decreased gradually, as if the stripping rate was higher than the fermentation rate. These results were the same as with the other studied fermentations, where stripped ethanol concentration start to decrease at the same time as control ethanol reaches a plateau. This result with periodically stripped fermentation further backed up the fact that a depletion of essential nutrients or an accumulation of other toxic materials stopped ethanol production.

5.2.3 Effect of CO_2 stripping on sugar consumption and fermentation rate

The reduced ethanol concentration in the stripped beer was expected to encourage yeast metabolism. As anticipated, CO_2 stripping resulted in higher sugar uptake in all the fermentation sets. Stripping of the standard 1080 and 1100 fermentations resulted in 5% and 9% increase in the consumption of total fermentable sugar respectively. The improved sugar uptake was attributed to a reduction of yeast inhibition via removal of ethanol through stripping. These results are in agreement with those of Groot *et al.* [1989] and Ennis *et al.* [1986] who found that continuous removal of butanol from ABE fermentations enhanced sugar uptake.

5.2.3.1 Selective maltose uptake with stripping

The higher sugar uptake in the stripped beer was primarily due to a higher maltose uptake. Indeed, in both control and stripped fermentations, the consumption of glucose, fructose and sucrose was almost identical and complete (between 90 and

96% of these sugars was consumed by day 14 of the fermentation), compared to maltose which remained in high proportion in the final medium. Maltose, whose initial concentration (94 and 113 g/L in the OG 1080 and OG 1090 fermentations respectively) was approximately twice the concentration of glucose (42 and 55 g/L respectively), was the least consumed. This was expected, as reported by Stewart *et al.* [1988] and Crumplen *et al.* [1989], only when 60% of the wort glucose is taken up by the yeast does the uptake of maltose commence.

5.2.3.2 Selective effect greater if non-agitated and non-aerated

The difference in sugar uptake between the control and stripped medium was greater for the non-aerated and non-agitated fermentations, as their control sugar consumption was relatively lower than in the standard fermentations. There was a 13 % decrease in sugar uptake in the non-agitated stripped fermentation (130 $g.L^{-1}$ initial volume) compared to the standard 1100 stripped fermentation (147 $g.L^{-1}$ initial volume). This is in accordance with the difference in medium ethanol level found between these fermentations. In the non-aerated stripped 1100 fermentation, as found for the ethanol production, the sugar uptake was not affected by aeration. As much as 151 $g.L^{-1}$ initial volume was consumed compared to 147 $g.L^{-1}$ initial volume in the Standard 1100 stripped fermentation.

5.2.3.3 Higher fermentation rates with stripping

The increase in total fermentable sugar uptake was confirmed by the higher fermentation rate in the stripped fermentations. The rate of fermentation was followed by the measure of the medium specify gravity. The specific gravity of the stripped fermentations was corrected for the volume loss due to the condensate and trap (when used), which resulted in a decrease of the measured specific gravity. In the standard fermentations and non-agitated fermentations with OG 1100, the additional trap at –40°C resulted in a higher correction than with the other fermentations, where only a condensation at 0°C was used. When the correction was applied with the fermentations operated with the additional trap, the rate of fermentation of the stripped medium was faster than in the control medium. These results were in agreement with the increased consumption of sugars found in the stripped fermentations compared to the controls. In the preliminary work using high gravity

cider medium (OG 1090), Huxtable [1993] found a slight increase in the rate of fermentation along with an increase in net ethanol production and sugar consumption. Contradictory results were found with the standard gravity (OG 1068) cider fermentation, where the rate of fermentation decreased with decrease of net ethanol production and increase in sugar consumption.

5.2.4 Effect of CO_2 stripping on net ethanol production

The higher sugar uptake and higher rate of fermentation was expected to be related to a higher net ethanol production, calculated from adding condensate and/or trap ethanol to medium ethanol. However, net ethanol production in stripped fermentations, was lower than in control fermentations. For example, in the standard fermentation with OG 1100, net ethanol production was 50 $g.L^{-1}$ initial volume in the stripped medium compared to 66 $g.L^{-1}$ initial volume in the control. Similar results were found for the other fermentations.

5.2.4.1 Water loss

Stripping led to a loss of volume and particularly a loss of water from the medium, creating a concentration of the fermentation medium and therefore an increase in medium osmotic pressure. As a first approximation, a decrease in ethanol production could have been related to an increase in osmotic pressure, as it has been reported that an increase in osmotic stress leads to a decrease in ethanol production and in fermentation rate (Crumplen *et al.* [1990]). During stripping of cider fermentations, Huxtable [1993] observed an increase in glycerol production in response to osmotic stress. In fact, when CO_2 was recirculated without condensation of the gas stream (the whole extracted condensate vapour was recirculated back into the fermenter), there was no increase in glycerol production. In the present study, the decrease in stripped medium ethanol from day 10-13 (where ethanol extraction rate is higher than its production rate), was probably due to an increase in osmotic stress. As suggested by D'Amore [1992], decreased fermentation activity under this osmotic stress was probably related to nutrient limitations. To verify the effect of osmotic pressure on the fermentation performance, the loss of volume by stripping could have been adjusted by the addition of sterile water. However, this operation would have added the risk of contamination.

5.2.4.2 Incomplete condensation

Unfortunately, results on the net production of ethanol are inconclusive due to the inefficiency of the condensation system (Chapter 4). The measured net ethanol production did not represent true ethanol production but only apparent net ethanol production. The condensation system was inefficient, as it did not capture all the volatile compounds extracted from the medium by stripping. It was shown that only 24% of the extracted ethanol was captured by the condensers (estimated from UNIFAC calculations). If this estimation is used to allow for the ethanol loss, estimated net ethanol produced would be equal to 109 $g.L^{-1}$ in the standard stripped fermentation with OG 1100 and to 95 $g.L^{-1}$ in the non-agitated stripped fermentation with OG 1100. If the measured consumed sugars were completely converted into ethanol, only 85 $g.L^{-1}$ and 73 $g.L^{-1}$ of ethanol respectively could be produced in theory. The estimated ethanol production using UNIFAC was, therefore, 29% greater than the maximum theoretical ethanol production. The estimation of ethanol loss with UNIFAC due to the inefficiency of the condensers is only applicable to synthetic mixtures where the estimation is accurate. Application of the results of synthetic stripping experiments to real fermentation broth is not accurate due to the presence of suspended biomass, acids, biological surfactants, antifoam, proteins and dissolved ions in the broth. The presence of some of these constituents, especially ions, in the liquid phase may decrease the ethanol partial pressure due to decreased activity. It was therefore not possible to determine a true net ethanol production.

5.2.4.3 Inhibitory effects

Ethanol removal from the medium through stripping was expected to remove ethanol inhibition, in order to allow the yeast to continue to ferment and therefore to enhance ethanol production. Ennis *et al.* [1986] and Groot *et al.* [1989] found that reduction of product inhibition by in-situ gas stripping increased butanol production in the ABE fermentation by *Clostridium acetobutylicum*. Huxtable [1993] found a reduction in net ethanol production in stripped cider fermentations with OG 1068. However, with higher original gravity cider fermentation (OG 1090), an increase in net ethanol production from 10.5% v/v (control) to 12% v/v was found in the stripped medium (Scott and Cooke [1995]). In those fermentations, the CO_2 stream produced by the fermentation was recirculated through the fermentation and not released into the

atmosphere (as is the case in this study). If not all the extracted volatile compounds were condensed, which is believed to be the case as the condensers were operating at -4°C, no loss of material should have occurred as the system was assured to be leak-proof. It was suggested that the higher sugar content of the high gravity fermentation, despite CO_2 inhibition and the osmotic stress, was a stimulating factor for ethanol production and improved fermentation rate.

5.2.5 Effect of CO_2 stripping on yeast physiology and morphology

5.2.5.1 Changes in environment conditions

With application of gas stripping, the yeast will be subject to changes in environmental conditions, in particular from increased exposure to CO_2. Scott and Cooke [1995] reported a rise in dissolved CO_2 from 0.10% w/w in the control to 0.37 % w/w in the stripped cider fermentation (OG 1068, 20°C) operating with an overpressure of $5x10^4$ Pa. Due to the rise in dissolved CO_2, medium pH fell sharply to a minimum of 2.8 compared to 3.15 in the control medium. In this study, dissolved CO_2 concentration was not measured directly, however the pH profiles of the stripped fermentations compared to the control fermentations were not adversely different. Only a slight but consistent decrease of 0.1-0.2 pH unit was observed in the stripped medium compared to the control medium at the end of the fermentation. As the fermentations were run under atmospheric conditions, there should have been only a minimum pressure build up, if any, and therefore negligible increase in dissolved CO_2. The main reason for the slight and consistent decrease in pH, was probably due to the fact that CO_2 solubility is inversely proportional to ethanol concentration. Relative CO_2 solubility has been reported to increase from 0.69 to 0.82 when ethanol concentration decreased from 10 to 5 % v/v (Jones and Greenfield [1982]). The decrease in stripped ethanol concentration (4.5 % w/w compared to 8.5 % w/w in the control) would therefore enable a slight increase in dissolved CO_2 and in medium pH.

5.2.5.2 Effects of dissolved CO_2

Depending upon concentration, CO_2 in beer medium has been reported to be either stimulatory or inhibitory. Yeast growth and metabolism has been shown to be stimulated by low concentrations of CO_2 up to a partial pressure (pCO_2) of $3x10^4$ Pa

(King and Nagel [1975]), probably due to CO_2 acting as a substrate in several essential metabolic biochemical reactions. Higher pCO_2 levels can be detrimental to the fermentation, and the actual inhibitory level is related to factors that influence gas solubility. For example, inhibition is increased by the presence of solutes such as ethanol and glucose (Jones and Greenfield [1982]). Cell size has also been found to increase at levels of $2.9x10^5$ Pa (Slaughter [1989]). Fusel alcohols and esters concentrations have also been found to reduce under elevated CO_2 pressure (Knatchbull and Slaughter [1987], Renger *et al.* [1992], Kruger *et al.* [1992]). In the present study, it can only be anticipated that the slight increase in dissolved CO_2 would probably have a positive effect on the fermentation. Indeed, it was found that yeast growth was improved in the stripped beer compared to the control. From the day when stripping was initiated (day 3), the number of suspended cells was higher in the stripped fermentations than in the control. The same result was obtained when the cell number was measured at the end of the fermentation after vigorous mixing of the medium, to enable suspension of the sedimented cells. Between 50 and 61 fold increases of stripped cells were observed compared to 24-31 fold increases of control cells in the fermentations with OG 1100. Stripping did not have the sole effect of keeping the cells in suspension (by the vigorous agitation effect), but indirectly improved cell growth. In addition to cell growth, cell viability was also improved. The decrease in cell viability in the stripped medium was less pronounced than the decrease in the control medium. Between 20 and 51% of cells were more viable in the stripped medium compared to the control. Enhanced yeast growth and cell viability was attributed to the removal of toxic ethanol through stripping, keeping the ethanol level under the inhibitory level.

5.2.5.3 Relative effects of CO_2 and osmotic pressure

The increase in cell growth and viability contradict conclusions of other workers on the effect of increasing osmotic pressure, which is usually related to a decrease in cell growth, cell viability and fermentation rate (Crumplen *et al.* [1990]). The increase in cell growth and viability in the present study was related to the decrease in ethanol inhibition, which probably had a greater effect on the yeast cells than increasing the osmotic pressure. In other words, the detrimental effect of osmotic stress was overcome by the stripping of ethanol from the medium. Huxtable [1993] reported

opposite results with the higher gravity cider fermentation (OG 1090) as cell growth and viability decreased in conjunction with an increase in ethanol production. In the higher gravity fermentation (OG 1090), exposure of the yeast cells to a higher concentration of CO_2 (as measured) had a greater effect on the yeast cells than the removal of ethanol inhibition. Despite the detrimental effect of CO_2 an increase in isoamyl alcohol production by 17% was observed in high gravity cider fermentation (Scott and Cooke [1995]). This contradicts the findings of Knatchbull and Slaughter [1987], Renger *et al.* [1992] or Kruger *et al.* [1992] who reported a decrease in higher alcohols under elevated pCO_2. This proves that more research is needed to elucidate the effects of dissolved CO_2 under the conditions of continuous stripping. In the present work, no contradictory results were found as the increase in yeast growth and viability, complements well the findings on increase rate of fermentation and improved sugar consumption. It is postulated that the slightly higher dissolved CO_2 was probably at a stimulating concentration (below inhibitory level), and that the main effect of stripping was due to reduction of medium ethanol.

5.2.5.4 Cell morphology

Cell size and cell biomass were also affected by CO_2 stripping. In the very high gravity fermentations (OG 1100), stripped cell size distribution shifted towards a greater number of larger cells. The increase in cell size could be attributed to the increase in osmotic pressure, as it has been reported that high osmotic pressure leads to plasmolysis of yeast cells (Jones and Greenfield [1982]). It was also found that biomass, measured as the cell mass in g per cell, increased in the stripped fermentations with fermentation time. This increase in biomass could therefore account for some increase in sugar uptake in the stripped medium.

The increase in cell size was also confirmed on SEM pictures, which also showed that as the size increased, the cells changed from an ovoid shape to a circular shape. There were no abnormal cells such as the ones described by Huxtable [1993], where a dramatic elongation of the stripped cells together with the formation of chains was observed. In the present work, slight elongation of cells was observed but randomly in the stripped and control medium. As *Saccharomyces cerevisiae* is a not a chain forming species, it is therefore surprising to find chains in Huxtable's fermentation

medium. It is not possible to totally reject the idea of a pseudomycelia appearing within the yeast cells as the cider fermentations were carried out in tower fermenters, where the overpressure reached up to $5x10^4$ bar. In the present work there was minimal pressure build up, therefore less stress on the yeast. However, the combined parameters (dramatic change of shape and appearance of a chain forming species) together seemed a doubtful coincidence. It is therefore possible to suggest that the odd cells found by Huxtable [1993] were possibly spoilage microorganisms rather than *Saccharomyces cerevisiae*, as possible contamination by oxidation was also pointed out.

5.2.6 Effect of CO_2 stripping on flavour compounds production.

As CO_2 stripping is not a selective separation technique, other volatiles, including the fusel alcohols are removed along with ethanol and water. As observed with ethanol, stripping reduced the concentration of isoamyl alcohol, isobutanol, propanol, acetaldehyde and ethyl acetate in the medium.

5.2.6.1 Higher alcohols

Within one fermentation, the extents of decrease of the higher alcohols in the stripped medium (concentrations not corrected for volume loss) were in the same order of magnitude. The three higher alcohols were reduced by 40-47%, 38-39% and 51-56% in the intermittent stripping 1080, non-aerated 1100 and standard 1080 fermentations respectively. In the standard 1100 fermentation, the three compounds were decreased by 70-72%. The volatility (as expressed by their dimensionless Henry's constant at 16°C, Chapter 4) of propanol, isoamyl alcohol and isobutanol are 0.200, 0.250 and 0.346 respectively. Due to the low difference in volatility, the extraction efficiency of those higher alcohols were expected to be in the same order of magnitude.

5.2.6.2 Ethyl acetate and acetaldehyde

Ethyl acetate, whose volatility (Henry's constant of 0.952) is higher than the later three higher alcohols, was completely removed from the beer medium by the end of the fermentation. Acetaldehyde which is the most volatile compound (Henry's constant of 3.159) was still present at the end of the stripped fermentation at

approximately 6-7 $mg.L^{-1}$ in both the standard 1100 and the non-aerated 1100 fermentations (it was not measured in the other fermentations). This possibly shows that the production rate/extraction rate ratio was higher for acetaldehyde than for ethyl acetate. Acetaldehyde is a branching point during ethanol production. It has been found to increase in high gravity brewing, due to inhibited yeast propagation and lack of sufficient dissolved oxygen (Hough *et al.* [1992]). As acetaldehyde is detrimental to the flavour of beer at high concentration, its reduction via gas stripping would therefore be beneficial to high gravity brewing. For example, stripping could be activated at specific times during the fermentation where the production is at its maximum, to reduce the level of acetaldehyde. Intermittent stripping would also be beneficial for the removal of other unwanted compounds such as sulphur compounds.

5.2.6.3 Net flavour compounds production

In high gravity (OG 1090) cider fermentation, Scott and Cooke [1995] found that despite the detrimental effect of CO_2 an increase in isoamyl alcohol production by 17% was observed. In this work, due to the negligible effect of CO_2, it was therefore expected to observe an increase in some of the beer volatiles. However, the measured net production of all these compounds was lower in the stripped fermentations than in the control fermentations. As found for ethanol production, the volatile compounds extracted by stripping were only partially captured by the condensation system. The relative apparent production of isoamyl alcohol, isobutanol and propanol in the stripped medium compared to the control medium was markedly higher than that of ethyl acetate and acetaldehyde. The relative apparent production of ethyl acetate and acetaldehyde varied between 1.2 and 11.5 % and between 33 and 39% of that of the control respectively. Due to their lower volatility compared to esters and aldehydes, the higher alcohols were less extracted and more condensed than the other two class of compounds. The relative apparent production of the three higher alcohols followed their order of volatility. As an example, in Standard 1080 fermentation, the relative apparent production of propanol, isoamyl alcohol and isobutanol were 92, 70 and 56% of that of the control respectively.

5.2.6.4 Apparent true production estimates

Percentage recovery by the actual condensation system (estimated with stripping of synthetic mixtures, Chapter 4) was used to estimated the "true" production of isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde in the stripped medium of Standard 1100 fermentation. Using these data, it was found that stripping produced a 1.5, 1.8 and 1.6 fold increase in isoamyl alcohol, isobutanol and propanol respectively, a 2-fold increase in ethyl acetate and a 6-fold increase in acetaldehyde. Although the values seem plausible for isoamyl alcohol, isobutanol, propanol and ethyl acetate, the 6-fold increase for acetaldehyde is more questionable. At the concentration of 111 $mg.L^{-1}$ (found after correction with percentage recovery by the condensation system), acetaldehyde would probably be considered as an off-flavour. Despite the interesting information given in Chapter 4 on the volatility of the different beer volatiles and the assessment of the condensation inefficiency, the results on net production of the main beer volatiles are inconclusive. As shown with ethanol, the estimation of the volatile compounds loss with UNIFAC due to the inefficiency of the condenser can only be applied to synthetic mixtures and not to real fermentation media. Total recovery of all the volatile compounds would involve a relatively large investment in the design of the condensation system. In a large-scale operation, there would be no need for such an investment, as the stripping gas would be recirculated in a closed system, where no loss of material would occur. As shown by Huxtable [1993] and Scott and Cooke [1995], where naturally evolved CO_2 was used, the increase in condensation temperature from –4°C to + 4°C resulted in a decrease in the amount of volatile condensed and therefore in a higher medium volatiles levels. No loss of volume was reported. The major drawback in such an operation, is the detrimental effect of dissolved CO_2 in the stripped medium, probably due to the length of the exposure rather than the level of CO_2 (as this was observed when the CO_2 was recirculated for more than 20 days during the fermentation). The designs of condensation units have not been usually reported by researchers in the field of gas stripping. Ennis *et al.* [1986], however, described the use of a 20 L condenser apparatus containing a cold finger filled with solid CO_2 (approximately –60°C), where the stripping gas (N_2) flow rate was 3.24 $L.min^{-1}$ (2.7 $L.L^{-1}min^{-1}$ for a 1.2 L fermenter). The percentage recovery was reported to be approximately 100%. This system is not suitable for a 15 day run of beer fermentation as a solid CO_2 trap

requires regular refilling. A possible way of increasing recovery efficiency without increasing investment cost would be to add columns packed with activated carbon in line after the condenser at 0°C, as used by Walsh *et al.* [1983].

5.2.6.5 Condensate analysis

The condensate and trap collected during stripping were analysed in terms of their content in the different beer volatiles. It was found that the changes in isoamyl alcohol, isobutanol, propanol, ethyl acetate, phenyl acetate, isoamyl acetate and acetaldehyde followed a similar trend to that of ethanol during the fermentation, which suggests that production of those volatile compounds was closely related to the production of ethanol. After being decreased in the first few days of stripping, the volatile concentration increased to (or stabilises at) a maximum during days 10-13 before being reduce by further stripping. This trend was more obvious for some volatile compounds than for others. As for ethanol, the increase in condensate concentration was related to a higher production rate than the extraction rate in the stripped medium. And the decrease in condensate concentration from day 10-14 was attributed to the reverse. The decrease in production at the end of the fermentation was related to a decrease in yeast fermentative activity, due possibly to increased osmotic pressure (which was not compensated by a supply of nutrients). This effect was greatly marked with ethyl acetate and acetaldehyde. Despite the high volatility of acetaldehyde, both condensate (and trap) and medium acetaldehyde increased sharply to reach a maximum on day 10, which suggests a high production rate of this compound.

Furfuryl alcohol and phenyl ethanol, which were the least volatile of the compounds studied in the present work, showed a different pattern in terms of changes in condensate concentration during the fermentation. Their concentrations were very stable (with a slight increase for furfuryl alcohol) through the fermentation and did not decrease towards the end of the fermentation. This suggests that the production rate of these compounds was always higher than the extraction rate, and that these two compounds did not suffer from a decrease in yeast fermentative activity towards the end of the fermentation, as suggested for ethanol and the other volatiles. Removal

of compounds such as ethanol and other volatile by-products, could shift yeast metabolism towards the production of other by-products.

5.2.7 Analysis of the beer flavour profile by ATD-GC/MS

Direct injection of the beer samples onto a packed column (as described in Chapter 3), enabled only the identification and the quantification of the main beer volatiles isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde (section 5.2.6). A more sophisticated technique enabling the concentration of the beer volatiles prior to injection onto the column, and the use of a capillary column was required for the identification and quantification of the minor compounds. The technique of Automated Thermal Desorption combined with GC/MS was used to qualitatively analyse the beer flavour compounds. Due to the lack of technical, economical and time resources, an extensive development of the method was not under the scope of this project. However, preliminary results using that technique indicated that it was possible to differentiate between stripped and control beers. Although the identification of the volatile compounds was not complete, due to insufficient analyses, the main higher alcohols, esters, acids as well as glycerol were identified. The mass spectra obtained for the various compounds are shown in Appendix G and compared with the one of the mass spectral database. It can be noted that the compounds identified in the beer medium with the GC/MS technique can not be directly compared with the fermentation sets presented in this study. The development of the technique was carried out at an early stage in this research project, with beer processed under different fermentation conditions. There was unfortunately no analysis performed on the condensate at this stage to back up the following findings.

When the chromatograms were compared in terms of peak area ratio, it was found that some compounds were reduced or totally removed from the medium when stripping was operated. The stripped beer volatile profile showed an imbalance of some compounds compared to the control. This study complemented the previous findings that stripping reduces the level of many compounds such as the fusel alcohols and the esters, as shown in section 5.2.6, but it also promoted the production of some other by-products. Compounds such as ethyl pentanoate were identified in

the stripped medium but not in the control medium. Removal of toxic ethanol and other volatile compounds could alter yeast metabolism, which would drift towards the production of other by-products. As this is only a preliminary study, further work needs to be carried out to identify accurately changes in flavour active compounds between the stripped and control beers. The thermal desorption-GC/MS technique has been proved to be very efficient for the analysis of beer flavour profile (Chen [1983], Kaipainen [1992]), and can be directly applied to the qualitative differentiation of stripped beer over control beer.

However, the main interest for this study is the quantitative analysis of the beer medium processed under different conditions, to enable the detection of a potential change in the production of flavour compounds when stripping is used during the fermentation. For this purpose, the ATD-GC/MS technique is not as straight forward as many parameters need to be controlled. The gas flow rate of the purging gas (N_2) needs to be the same for both the stripped and control beer. The flow rate was limited to 3-5 $mL.min^{-1}$ in the control beer due to excessive foaming. In the stripped beer, which has been already treated with CO_2, a flow rate up to 25-30 $mL.min^{-1}$ was possible. An antifoam could be added to the sample to counteract this problem. In addition, the difference in ethanol content between the control and the stripped beer would also alter the stripping efficiency of the different volatile compounds. The quantification of the beer flavour compounds would also need the preparation of a calibration curve for each of the compounds. The extraction efficiencies of standard compounds from the synthetic mixtures would be different to the ones from the complex beer medium. The use of internal standards (whose volatility range from the lowest to the highest volatility of the beer flavour compounds) would be useful to minimise the errors, due to a difference in ethanol concentration and in medium composition (compared to synthetic mixtures). As it was found that the analysis of sulphur compounds would be useful, a specific detector such as the NPD detector would be required (Leppänen *et al.* [1979]). It is clear that more development work is required to quantify accurately the difference in production of flavour compounds between control and stripped beer. For accurate measurement of the net flavour production both medium and condensate/trap should also use the same analytical technique.

5.2.8 Condensate/trap concentration using pervaporation

Dilution of high gravity beer is usually carried out in order to obtain the desired ethanol concentration. However, due to the removal of higher alcohols, esters and acids, the stripped beer would lack flavour. It would therefore be necessary to add back some of these compounds to the level normally found in standard beer.

The condensate and trap collected from the stripping of high gravity beer fermentation, represent a source of natural flavour compounds. Their concentration in the condensate and trap were much greater than in the fermentation medium. The higher alcohols were between 2 to 6 times more concentrated in the condensate than in the medium, and between 14 to 17 times more concentrated in the trap than in the medium. The condensate was enriched in the least volatile compounds (such as furfuryl alcohol and phenyl ethanol) and the trap in the most volatile compounds (such acetaldehyde, ethyl acetate, and ethyl caprylate). If further concentrated and fractionated, selected compounds could be added back to the finished beer to counterbalance the organoleptic quality of the finished beer. No exogenous chemical compounds would be needed as they could be provided by the fermentation itself. As additives are often regarded suspicious by the consumers, recovery of the condensate components would make use of the natural flavour compounds present in the beer originally so that no special declaration of contents would be necessary.

As the condensate or trap is a clean solution, its use as a feed for pervaporation studies was straight forward. The feasibility study was carried out with synthetic mixtures containing the main alcohols produced during the beer fermentation. Hydrophobic (organophilic) membranes were employed to allow the preferential permeation of the volatile organic compounds (VOCs). With an artificial condensate (containing approximately the concentrations found in real condensate) concentrations of ethanol, propanol, isobutanol and isoamyl alcohol were respectively 5, 14, 19 and 33 times higher in the permeate than in the feed. The results were also in the same order of magnitude as other data reported on recovery of aroma compounds from dilute solutions (Karlsson and Trägårdh [1993]). Further work should be carried out with esters and aldehydes found in the natural condensate. Bengtsson *et al.* [1990] found that alcohols had the lowest enrichment factors

compared to esters, which have been concentrated more than 100-fold, and aldehydes between 40 to 60 times.

The recovery of alcohols was greatly affected by the presence of ethanol as found by Karlsson and Trägårdh [1994]. When ethanol was at 100 $g.L^{-1}$ (12.7 %v/v) in the feed, the enrichment factor of propanol, isobutanol and isoamyl alcohol decreased by 8%, 74% and 77% respectively compared to a feed where ethanol was at the same concentration (at 500 $mg.L^{-1}$) as the other three components. The presence of ethanol adversely affected the activity coefficients of isoamyl alcohol and isobutanol, thus reducing the activity difference (and hence the component fluxes) across the membrane. The higher the hydrophobicity of the volatile component the greater the effect of ethanol was. It was also found that higher enrichment was obtained when the hydrophobicity of the membrane was increased for example by a high proportion of long chain alkyl groups. From a standard PDMS to a GKSS membrane, the enrichment factors of isoamyl alcohol, propanol, isobutanol and ethanol were improved by 35%, 39%, 46% and 50% respectively.

Pervaporation is an interesting alternative to existing flavour recovery processes, especially for the enrichment of a beer condensate obtained by CO_2 stripping of beer fermentation. Existing methods of flavour concentration include vacuum evaporation and reverse osmosis, which suffer from the loss of aroma compounds. Scott *et al.* [1996] and Scott *et al.* [1997] studied the recovery of volatile organic compounds from alcoholic beverages by high boiling point extractants (HBEs), such as 1-nonanal, heptanal, and oleyl alcohol. Despite good recoveries (100% for isoamyl alcohol), separation using HBEs, requires a second separation stage such as flash distillation, which could potentially result in thermal decomposition of some flavour compounds. Additionally, this technique is not as clean as pervaporation, as the concentrated flavour extract could contain traces of solvent, which would not be desirable if added back to the alcoholic beverage. With pervaporation, a two-stage condensation could be used to achieve a further separation of the beer condensate. The highest condensation temperature (for example 0°C) could be use to recover a fraction containing mainly water and ethanol, while the lower condensation temperature (for example –20°C) would captured the higher alcohols, esters and aldehydes.

CHAPTER 6 - CONCLUSIONS AND FUTURE WORK

6.1 CONCLUSIONS

Removal of ethanol and other volatile compounds by CO_2 stripping, followed by recovery by condensation is relatively straightforward in terms of processing equipment. In addition to the simplicity of operation, CO_2 stripping offers several advantages over existing extraction methods such as the low investment cost, no application of heat, and the option of continuous or periodic extraction.

An assessment of the volatility of various beer flavour compounds in synthetic mixtures was determined using both published data on Henry's constants and calculated K^{∞} values using the UNIFAC method. The degrees of volatility were determined for compounds identified in the beer fermentations studied such as the higher alcohols (i.e. isoamyl alcohol, isobutanol, propanol and 2-phenyl ethanol) and the esters (i.e. ethyl acetate, isoamyl acetate and ethyl caprylate). In general, the volatility of the esters was higher than for the higher alcohols. If needed by the brewer, the UNIFAC program would enable an approximate estimation of the relative volatility of other important beer flavours such as the fatty acids (i.e. propanoic acid, caproic acid and caprylic acid), the ketones (i.e. diacetyl and 2,3-pentanedione) and the sulphur compounds. This data provides useful information on how beer fermentations can be manipulated in terms of determining the expected relative removal of volatiles by gas stripping. It could therefore be exploited in the future for the extraction of beverage off-flavours, such as acetaldehyde, diacetyl or some sulphur compounds (when produced in high concentration). For example, by initiating short extraction during high concentration of those volatiles, an exaggerated removal of them could be achieved.

CO_2 stripping of high gravity beer fermentations (OG 1080, OG 1100) enabled medium ethanol concentration to be kept under the yeast inhibitory level (8.5 %v/v). It therefore resulted in an increase in sugar uptake, cell growth and viability. The increase in sugar uptake (primarily maltose uptake) was confirmed by a faster fermentation rate (as measured by the specific gravity). However, the fermentation of sugars was incomplete, as maltose remained at a relatively high concentration in the stripped medium. Although ethanol was kept under the toxic level, ethanol production rate started to decline from day 10-13 of the fermentations compared to its

extraction rate. Until 10-13 of the fermentation, removal of ethanol inhibition had a greater effect on the yeast cells than the increase in osmotic pressure, which was confirmed by the increase in cell growth and cell viability. However, after day 13, decrease in ethanol production rate was attributed to an increase in osmotic pressure (which could explained the increase in cell size and total biomass) and/or a depletion of essential nutrients and oxygen. In the future, adjusting the beer medium volume should be considered (for example by adding an amount of water equal to the amount of condensate being removed, under sterile conditions) so that the increase in osmotic pressure proportional to the removal of condensate would be avoided.

Despite a higher sugar uptake and fermentation rate (measured by the change in specific gravity), measured net ethanol and other flavour compounds production (neglecting vapour phase losses) was reduced. The results on net production of these compounds were inconclusive, as the recovery of the volatile compounds was incomplete. As estimated with stripping of synthetic mixtures, only 24% of ethanol was recovered. However, the application of the theoretical percentage recovery to the estimation of ethanol production resulted in a 29% increase compared to the theoretical maximum ethanol production (calculated from consumed sugars). If the respective percentage recoveries were applied to the main beer flavour compounds, then isoamyl alcohol, isobutanol, propanol, ethyl acetate and acetaldehyde production in the stripped beer could be estimated. The use of this estimation resulted in an increase in the production of these compounds compared to the control. As shown for ethanol, the application of the UNIFAC method (using simple mixtures) is not directly applicable to fermentation medium, and could result in erroneous conclusions.

The changes over time of most of the volatile compounds in the condensate closely followed those of ethanol, which shows that the production rate of those compounds was closely related to the ones of ethanol. The changes in concentrations of furfuryl alcohol and phenylethyl alcohol followed a different pattern (as the condensate concentration did not decrease after day 10-13), which possibly shows that their production was not affected by the increase in osmotic pressure nor by the depletion of essential nutrients. It could also mean that the removal of ethanol and other by-products of the fermentation, enable a higher production of other compounds. In other

words, CO_2 stripping could affect the yeast metabolism, which would drift towards the production of other by-products. This was confirmed by the use of a more sensitive analytical technique (ATD-GC/MS) for the analysis of medium volatile compounds. Not only was the whole flavour profile of the stripped beer different to the control beer (the level of most of the volatile compounds in the stripped medium was reduced), but other compounds such as ethyl pentanoate was detected in the stripped beer but not in the control beer.

CO_2 stripping has potential in terms of a dealcoholisation technique as first introduced by Huxtable [1993] and Scott and Cooke [1995] for cider fermentations. Although not the main objective of the present study, CO_2 stripping, as well as increasing the overall fermentation rate, enabled the production of a reduced alcohol beer (4.5% ethanol with OG 1100 at 16°C). A beer containing 4.5% alcohol is not strictly speaking a low alcohol beer. However, if diluted by 50%, as it is currently carried out with high gravity brewing, as low as 2.5 % v/v alcohol content could be achieved. The overall flavour of the diluted beer would require enhancing (as stripping reduced also the level of many other volatile compounds), which could be achieved by adding fraction of the beer condensate.

The extracted condensate is a source of natural beer flavour compounds. The concentration of the condensate by pervaporation resulted in enrichment of the volatile compounds such as the higher alcohols. The enrichment factors increased with decreasing feed (condensate) ethanol concentration and increasing membrane hydrophobicity. A two-stage condensation system could be used to further separate water and ethanol from the other volatile flavour compounds. A fraction of this permeate could be added back to the diluted stripped beer to counterbalance the flavour loss through stripping. The concentration and/or fractionation of the beer condensate by pervaporation imply that no exogenous compounds would be required by the brewer.

For large-scale operation, the naturally evolved CO_2, source of free extractant, could be recirculated through the medium. However, as found by Huxtable [1993], the relative high pressure would result in higher dissolved CO_2, which is inhibitory to yeast fermentative activity. Successful operation of gas recycle would require a

control of the system parameters (pressure, gas flow rate, temperature), so that removal of ethanol inhibition would counteract CO_2 inhibition.

The CO_2 stripping technique was proved adequate for the fermentation of high gravity wort by increasing the rate of fermentation. It suggests that, despite the observed partial condensation, stripping would increase net ethanol production as estimated using UNIFAC calculations. Providing that all the stripped ethanol is recovered, dilution of the stripped beer (process which is usually adopted by the high gravity brewing industry) would require the addition of condensate ethanol to achieve a typical beer strength. The flavour balance of the beer could be restored or enhanced by the addition of selected flavour compounds, provided by fractionation/concentration of the beer condensate. Ultimately, dilution of the stripped beer without any addition of ethanol could be of interest to the low-alcohol brewing industry.

6.2 FUTURE WORK

1) Gas stripping can be used to manipulate the production of beer flavour compounds. For this purpose, accurate quantification of these compounds would be required. The ATD-GC/MS technique, which proved to be adequate for identification of the different components should be further investigated for quantification purposes. If production of particular unwanted compounds (such as sulphur compounds, diacetyl or acetaldehyde) was found to reach a peak during the fermentation, stripping could be activated at specific times to exaggerate their removal. The analysis of sulphur compounds would require the use of a FPD detector.

2) Further studying of the gas stripping technique with exogenous CO_2 would require an improvement of the condensation unit, in order to enable a mass balance to be determined. First, it would enable the comparison of the absolute volatility of the compounds with theoretical data. Secondly, it would enable the accurate determination of the net ethanol and other flavour compounds production. For operation in a closed-loop system with CO_2 recycle (where CO_2 would be provided by the fermentation itself), improved condensation of the volatile compounds would also be advantageous, to improve the stripping efficiency, impaired by volatile-non

free CO_2. As found by Huxtable [1993], who used a closed system, an increase in the condensation temperature from –4 to +4°C, resulted in a decrease in the condensate concentration and a decrease in CO_2 extraction efficiency.

3) To avoid the effect of an increase in osmotic pressure towards the end of the fermentation, the medium volume should be constantly adjusted. For example, a volume of sterile water equal to the condensate volume should be added daily under sterile conditions during the fermentation. This would remove one of the factors which was found responsible to the decrease in ethanol production towards the end of the fermentation. If ethanol production was still not reactivated after compensating for the osmotic pressure, other factors such as the lack of essential nutrients or oxygen should be investigated.

4) In the present study, fermentation was completed in 15 days and was therefore slower than for a normal industrial brewing fermentation, which is normally completed within 10 days for a typical bottom fermentation or 3 days for a typical top-fermentation (Hough *et al.* [1982]). Due to the length of the fermentation, only a limited number of fermentation sets were carried out. The beer medium was prepared from a Home Brew kit. Further work should consider the use of a typical high gravity wort, provided for example by a local brewery. If this is not possible, further studies should consider to increase the rate and extent of the fermentations, by altering parameters such as the fermentation temperature, the wort oxygen level, and the yeast pitching rate (D'Amore [1992]).

5) Further analytical work should be carried out to accurately distinguish between the qualitative and quantitative profiles of stripped and control beer. These analyses should also be carried out in conjunction with sensory analysis of the finished beer, and the consumer preference assessed between the stripped and control beer when adjusted back to the same level of ethanol. Sensory analysis would involve the training of a panel, the screening of sensory descriptors and flavour standards, and the interpretation of the chosen mode of analysis (i.e. Quantitative Descriptive Analysis). This work combining analytical and sensory analysis constitute probably on its own the subject of long research study.

6) The preliminary study on concentration of the beer condensate by pervaporation could be extended by using a larger range of flavour compounds, as the higher alcohols were the only compounds considered. The use of a two stage condensation unit should also be investigated to further separate water and ethanol from the other volatile flavour compounds. For its potential use in the brewing industry, the economic validity of the technique should be evaluated in comparison with existing fractionation methods such as distillation.

CHAPTER 7 - REFERENCES

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Appendix A - NCYC 1236 specification

The specification of the NCYC 1236 were given as follows: "British brewery (1966). Non-flocculent. 3:3:5:5:3. O1, 8% alcohol tolerant, low pyruvic, high a-hydroxyglutaric, SG 5.5, pH3.95 ferments rapidly using maltotriose readily. High ergosterol, occasionally gives propagation trouble, needs new culture prepared frequently, possibly has a CO_2 requirement, 2 micron+. Max. Temp. 35°C, Min. Temp. 12°C, Optimum Temp. 33°C."

Most brewing strains have been assessed by means of the EBC fermentation tubes at the Brewing Research Foundation (Walkey, R.J. and Kirsop, B.H., 1969, J.Inst.Brew., 75, 393) and the information so obtained is provided in each entry (eg. 5:3:1:5:3). The numbers range from 1 to 5 and indicate the degree to which certain fermentation characteristics are exhibited, 5 indicating the highest degree of expression. The five characters tested are:

1) Head formation (the accumulation of yeast at the surface of the fermentation):

1 : no head, 3 : intermediate, 5 : good head.

2) Deposit (the amount of deposited yeast):

1 : < 8mm, 3 : 8-15mm, 5 : >15mm.

3) Attenuation (the degree to which fermentation occurs). If the lowest S.G. is 6.0, then attenuation at 6 days of 6-8 : 5, 8-10 : 4, 10-12 : 3, 12-14 : 2, 14-1 : 1

4) Rate of fermentation: If the difference between the S.G. at 3 days and at 6 days is: 0-2 : 1, 2-4 : 2, 4-6 : 3, 6-8 : 4, 8-10 : 5

5) Clarity of the final beer: If the nephelometer reading of a 1:20 diluted sample is 0-10 : 5, 10-20 : 3, >20 : 1.

Most brewing strains are recorded as being flocculent or non-flocculent. In addition, flocculent strains have been further classified as FLO1 type flocculation, new FLO type flocculation and chain forming yeast (M.Stratford, 1989, Yeast, 5, S441-S445).

*FLO1 type flocculation -- partially inhibited by mannose, resistant to inhibition by salts or low pH, usually constitutive.

*New FLO type flocculation -- completely inhibited by mannose, maltose, sucrose and glucose, more sensitive to salt/low pH inhibition, developed in stationary phase of growth, delayed or prevented by excess ammonium salts.

*Chain formation -- failure of buds to separate from mother cell, leads to clumps of 20-30 cells, mechanically separated clumps will not reform.

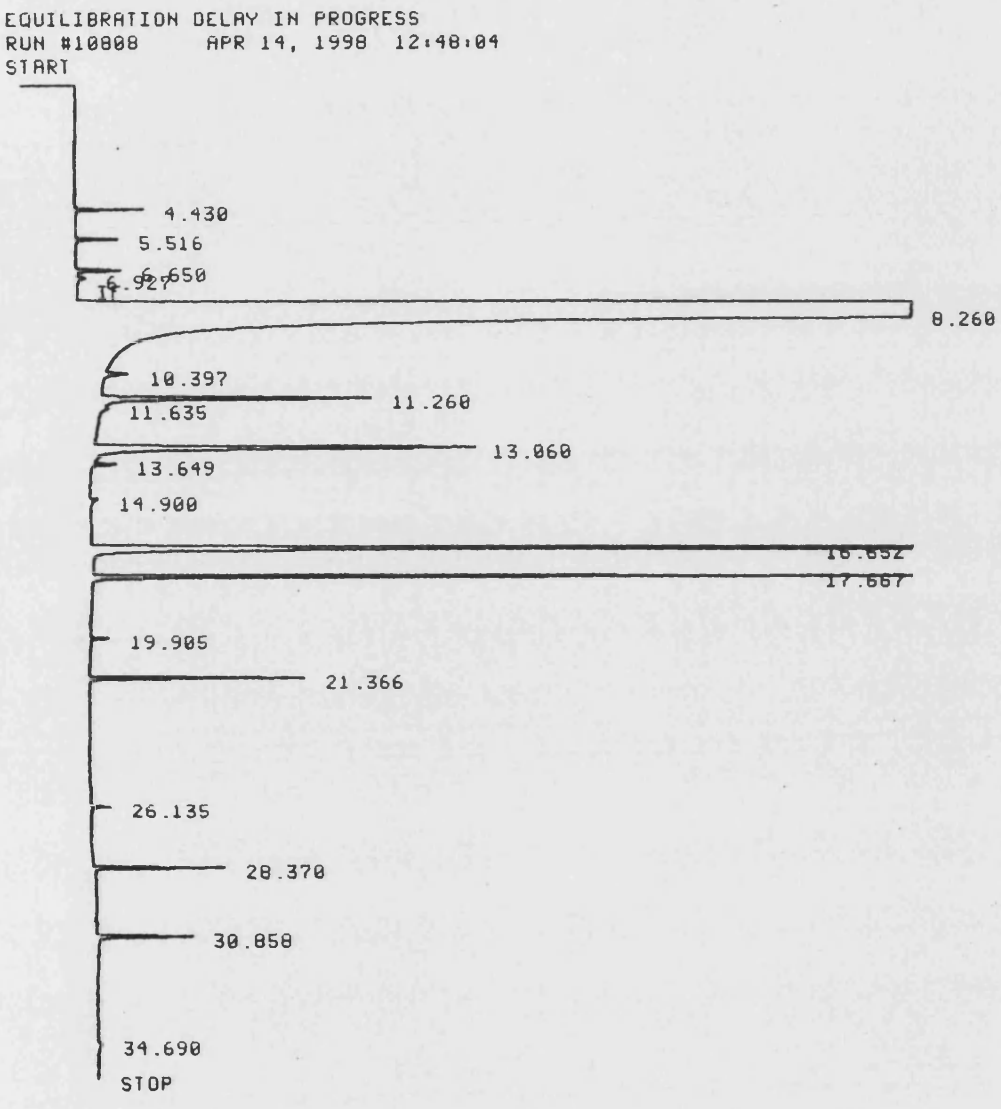
The growth temperatures were measured in a shaking gradient incubator at NCYC; maximum, minimum and optimum temperatures are shown where available.

The quantity given for DMS is the maximum dimethyl sulphide concentration reached in 72h in laboratory stirred fermentations (μg/l).

The oxygen Requirements were classified as follows:

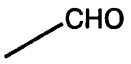
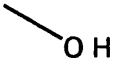
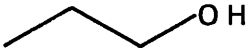
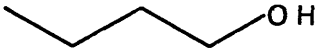
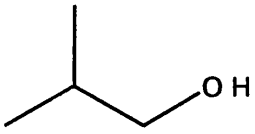
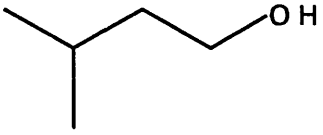
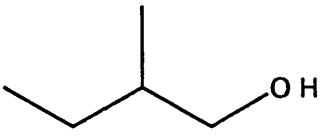
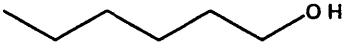
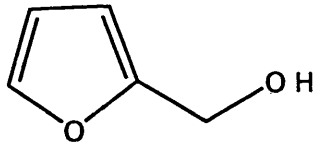
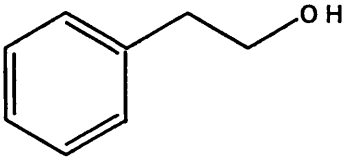
O1 : requirements met by half air saturated wort (4ppm).
O2 : requirements met by air saturated wort (8ppm).
O3 : requirement met by oxygen saturated wort (40ppm).
O4 : requirement not met by oxygen saturated wort (40ppm)

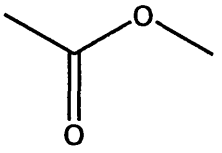
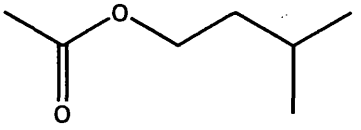
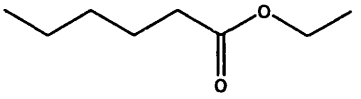
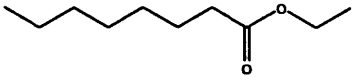
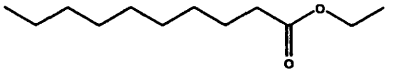
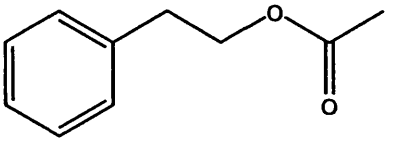
Appendix B - GC chromatogr am of a beer condensate



Compound	Retention time	Compound	Retention time
Acetaldehyde	4.430	n-butanol	14.900
Acetone	5.516	Isoamyl alcohol	16.652
Ethyl acetate	6.650	IS (1-pentanol)	17.667
Methanol	6.927	1-hexanol	19.905
Ethanol	8.260	Ethyl caprylate	21.366
Isobutyl acetate	10.397	Furfuryl alcohol	26.135
Propanol	11.260	2-phenylethyl acetate	28.370
Isobutanol	13.060	2-phenyl alcohol	30.858
Isoamyl acetate	13.649		

Appendix C - Chemical structure of some beer volatile compounds

Class of compound	Compound	Chemical structure
Aldehyde	Acetaldehyde	
Alcohol	Methanol	
	propanol	
	n-butanol	
	isobutanol	
	isoamyl alcohol	
	amyl alcohol	
	hexanol	
	furfuryl alcohol	
	2-phenyl ethanol	

ESTERS	Ethyl acetate	
	Isoamyl acetate	
	Ethyl hexanoate (caproate)	
	Ethyl octanoate (caprylate)	
	Ethyl decanoate (caprate)	
	2-phenyl ethyl acetate	

Appendix D - Analytical data

1) Concentration of sugars in malt and brewing liquid sugar

The concentrations of maltose, glucose, sucrose and fructose in the malt and brewing liquid sugar (Edme) were analysed using an enzymatic method (Chapter 3). The results agree with typical concentrations found in wort (Hough *et al.*[1982]).

	Concentration (% w/w)			
	Maltose	**Glucose**	**Sucrose**	**Fructose**
Malt	51	8	1.6	1.0
Brewing sugar	32	21	1.0	1.7

2) Maltotriose concentration

Maltotriose was not measured by the enzymatic method. The concentration used in the calculation of fermentation efficiency was estimated from information given by the sugar manufacturer and by Hough et al. [1982] for the concentration in the malt. In the malt, maltotriose ranged from 15.3 to 19.3 % of the total fermentable sugar for beer wort of original gravity ranging from 1040 to 1054. An average maltotriose concentration of 17% of total fermentable sugar was calculated over seven different beer types. This data was used to calculate the approximated amount of initial maltotriose in the wort.

	Maltotriose % w/v	**Source**
Malt	13	Hough *et al.* [1982]
Brewing sugar	7.4	Manufacturer

Appendix E - Estimation of th e sugar consumption curve

The concentration of the sugars maltose, glucose, fructose and sucrose in the beer medium was only analysed on day 0 and day 14 of the fermentation. To calculate fermentation efficiency, it was necessary to estimate the concentration of sugars removed by daily sampling of the fermentation medium. Therefore a sugar consumption curve was estimate from the knowledge of the change of ethanol production during the fermentation and of the sugar concentrations on both day 0 and day 14. It was estimated that the consumption of sugar followed the reverse of ethanol production, and was calculated as follows:

$$[maltose]_{dayi}=(([rev.\ ethanol]_{dayi}/[reverse\ ethanol]_{day0})*[maltose]_{day0}-[maltose]_{day14}) + [maltose]_{day14}$$

$$Where,\ [reverse\ ethanol]_{dayi}= [ethanol]_{day14} - [ethanol]_{dayi}$$

Figure 7–1 shows the estimated sugar consumption curve and the ethanol production curve for the standard fermentation with OG 1100.

The estimation of sugar consumption is clearly not accurate as the four main sugars maltose, glucose, fructose and sucrose doesn't have the same rate of consumption. Foe example, due to catabolic repression, maltose is only metabolised when 60% of glucose has been metabolised (Hough *et al. [1982]*). However, it gives a reasonably good evaluation of the sugar uptake during the fermentation. The fermentation efficiencies based on this estimation gave realistic values.

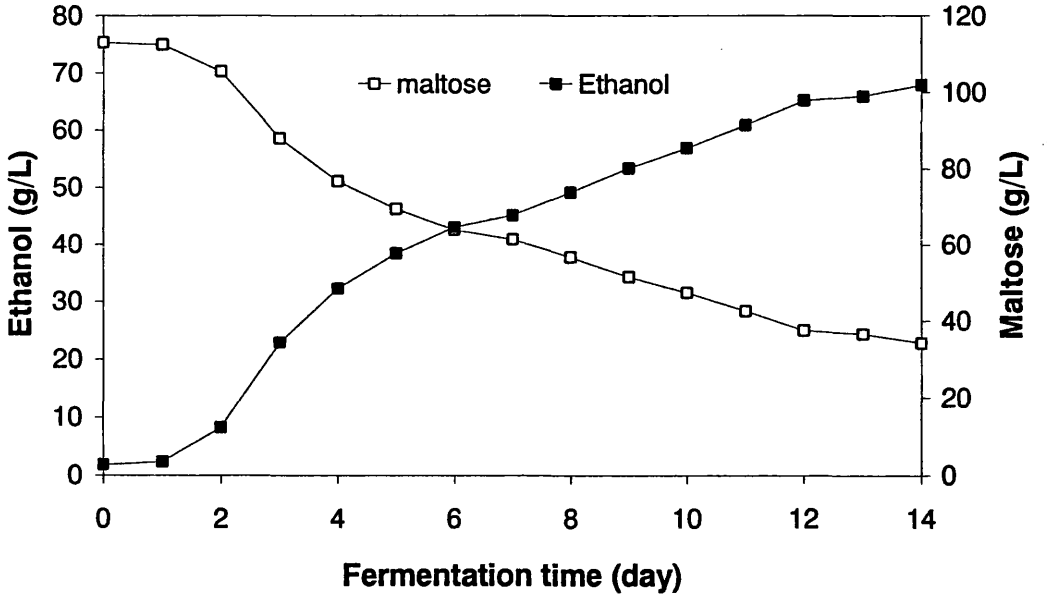


Figure 7–1: Changes in ethanol concentration and in sugar consumption (estimated from the ethanol production and sugar concentrations on day 0 and day 14)

Appendix F - Concentrations of sugars in stripped and control fermentations

Table 7-1: Maltose concentrations in stripped and control fermentations.

Fermentation set	Initial concentration (g/L)	Residual concentration (g/L initial volume)		Loss through sampling (g/L initial volume)		Fermented sugar (g/Linitial volume)		Residual concentration (g/Lfinal volume)	
		control	stripped	Control	stripped	Control	stripped	Control	stripped
Standard 1080	93.8	10.7	5.2	6.6	6.2	76.5	82.4	12.2	6.6
Standard 1100	113.1	30.4	19.8	6.3	5.4	76.4	87.8	34.0	25.8
Non-agitated 1100	113.0	56.2	31.4	10.8	9.6	46.2	72.1	64.7	41.9
Non-aerated 1100	113.1	52.0	10.8	11.7	8.8	49.4	93.5	62.2	15.1

Table 7-2: Glucose concentrations in stripped and control fermentations.

Fermentation set	Initial concentration (g/L)	Residual concentration (g/L initial volume)		Loss through sampling (g/L initial volume)		Fermented sugar (g/Linitial volume)		Residual concentration (g/Lfinal volume)	
		control	stripped	Control	stripped	Control	stripped	Control	stripped
Standard 1080	42.4	0.3	0.3	3.3	3.2	38.8	38.9	0.3	0.4
Standard 1100	54.7	0.4	0.4	2.1	2.0	52.2	52.3	0.5	0.5
Non-agitated 1100	54.7	0.2	0.3	2.9	3.0	51.7	51.4	0.3	0.3
Non-aerated 1100	54.7	1.1	1.0	2.8	2.8	50.8	50.9	1.3	1.5

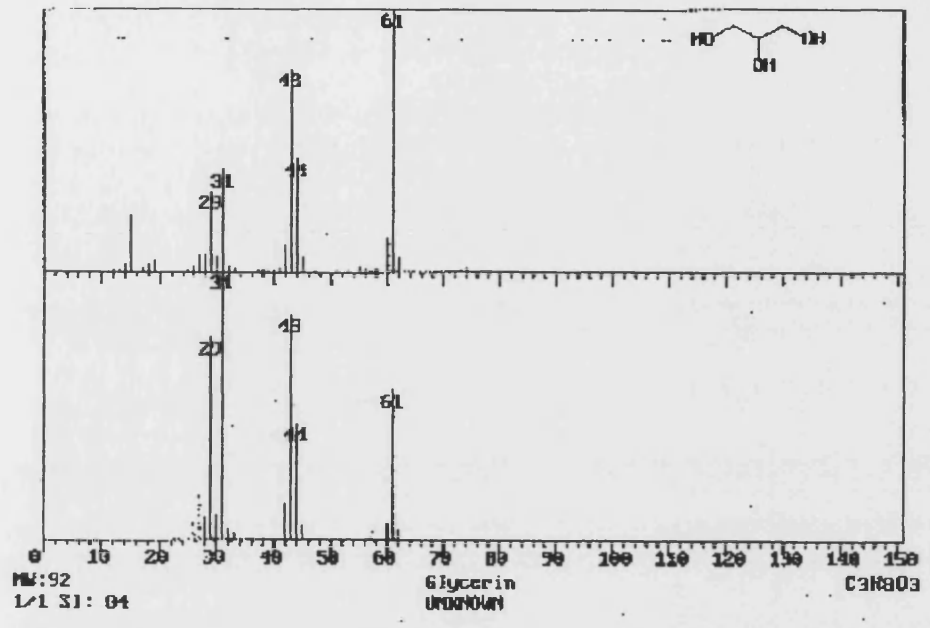
Table 7-3: Sucrose concentrations in stripped and controlfermentations.

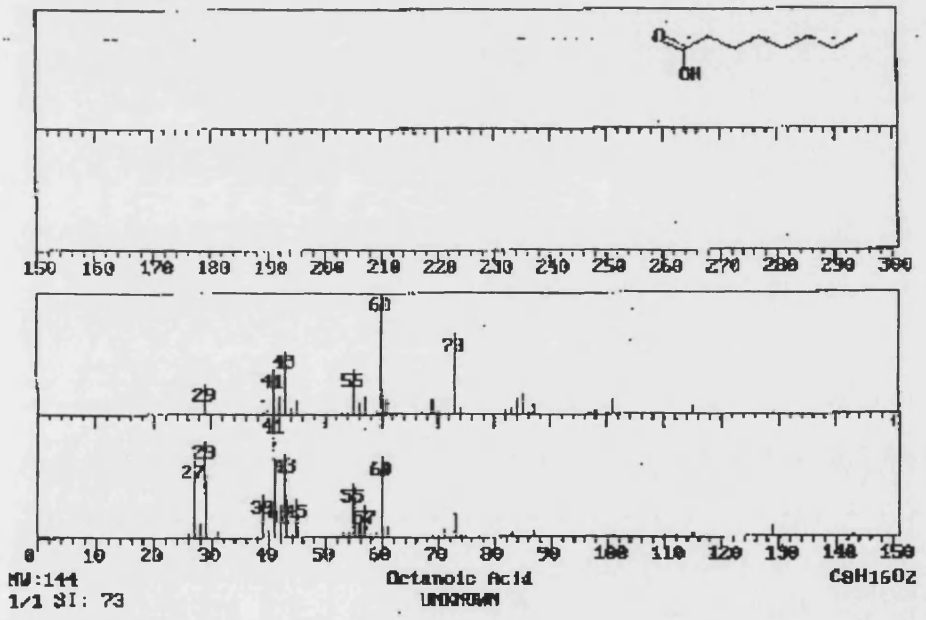
Fermentation set	Initial concentration (g/L)	Residual concentration (g/L initial volume)		Loss through sampling (g/L initial volume)		Fermented sugar (g/Linitial volume)		Residual concentration (g/Lfinal volume)	
		control	stripped	Control	stripped	Control	stripped	Control	stripped
Standard 1080	2.1	0.0	0.0	0.2	0.2	1.9	1.9	0.0	0.0
Standard 1100	2.4	0.0	0.0	0.1	0.1	2.3	2.3	0.0	0.0
Non-agitated 1100	2.4	0.0	0.0	0.1	0.1	2.3	2.3	0.0	0.1
Non-aerated 1100	2.4	0.0	0.0	0.1	0.1	2.3	2.3	0.0	0.1

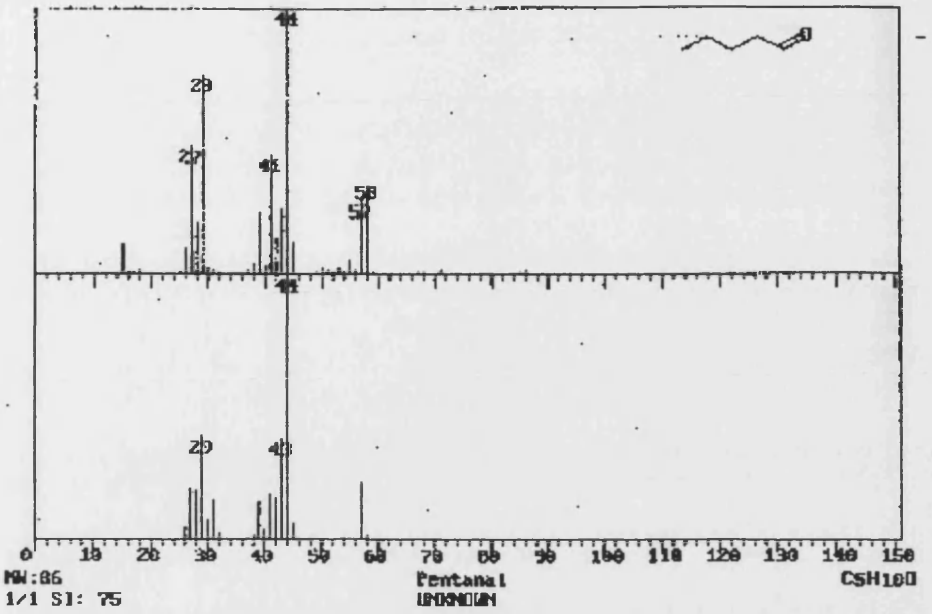
Table 7-4: Fructose concentrations in stripped and control fermentations.

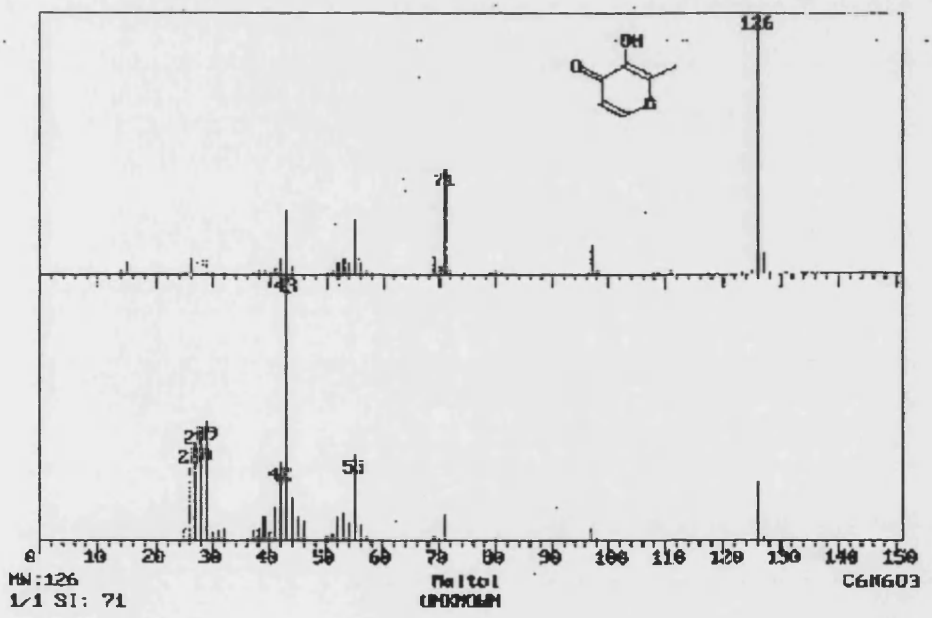
Fermentation set	Initial concentration (g/L)	Residual concentration (g/L initial volume)		Loss through sampling (g/L initial volume)		Fermented sugar (g/Linitial volume)		Residual concentration (g/Lfinal volume)	
		control	stripped	Control	stripped	Control	stripped	Control	stripped
Standard 1080	3.7	0.0	0.0	0.3	0.3	3.4	3.4	0.0	0.0
Standard 1100	4.7	0.0	0.0	0.2	0.2	4.5	4.5	0.1	0.0
Non-agitated 1100	4.7	0.0	0.0	0.2	0.2	4.5	4.5	0.1	0.0
Non-aerated 1100	4.7	0.1	0.1	0.2	0.2	4.4	4.4	0.1	0.0

Appendix G - Mass spectra

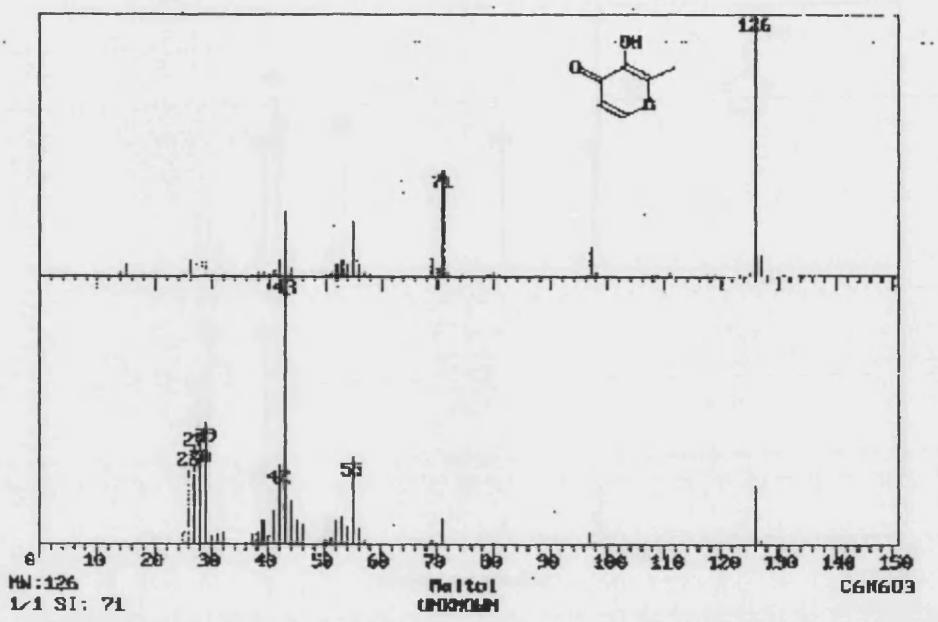


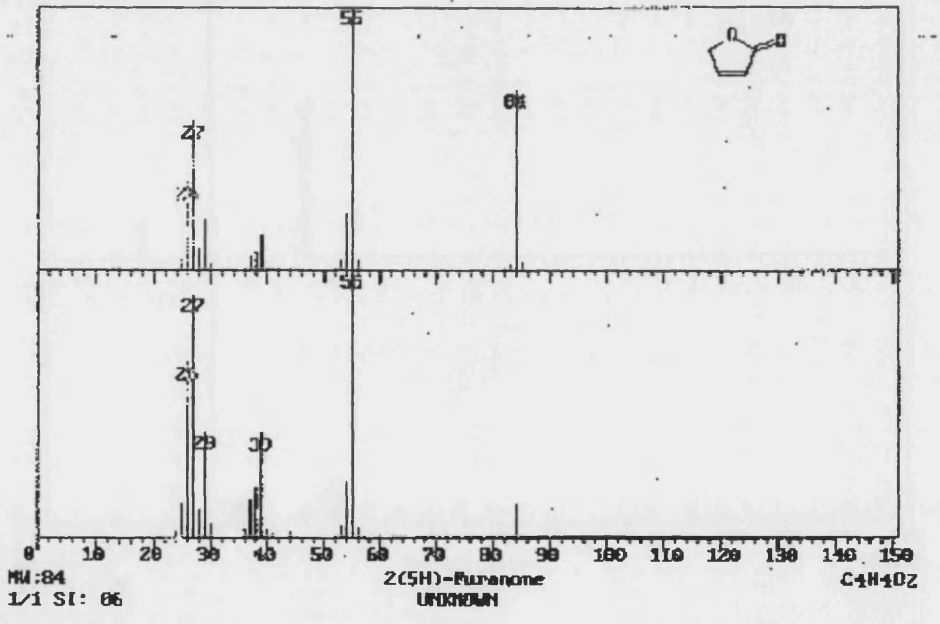


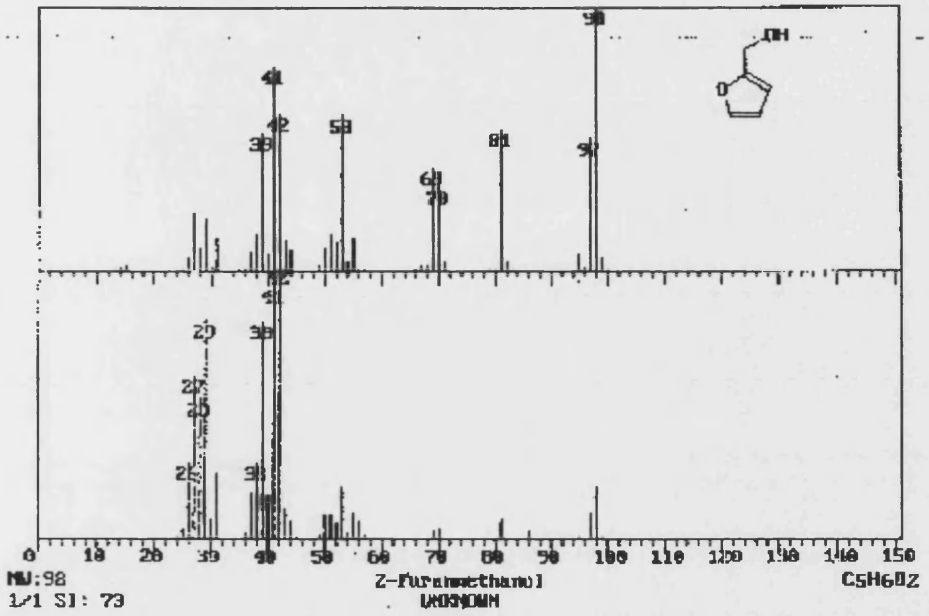

MW:86
1/1 SI: 75
Pentanal
C5H10O


MW:126
1/1 SI: 71
Maltol
C6H6O3

Appendix G


126
71
43
55
MW:126
1/1 SI: 71
Maltol
C6H6O3


55
84
27
56
MW:84
1/1 SI: 86
2(5H)-Furanone
C4H4O2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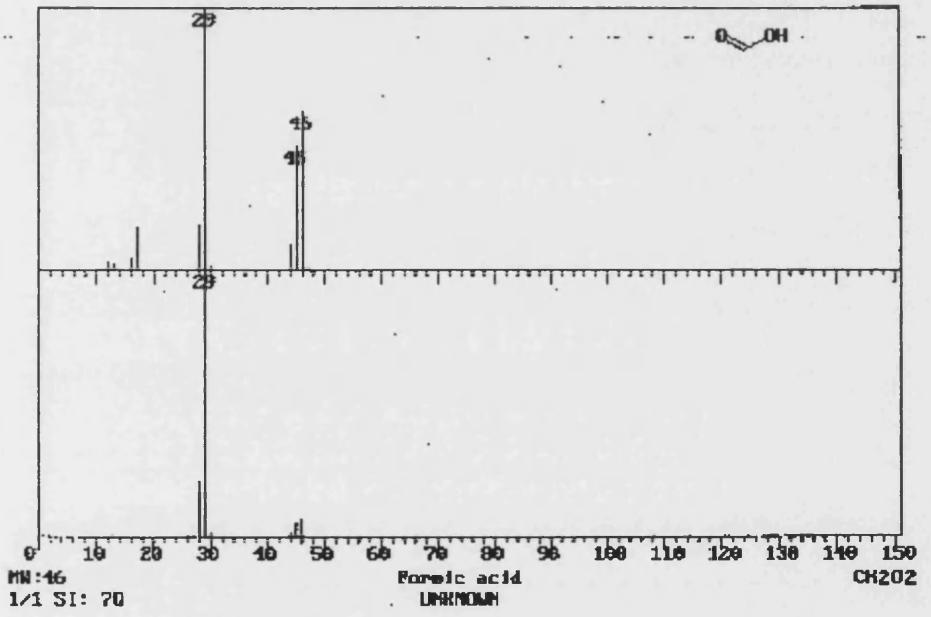
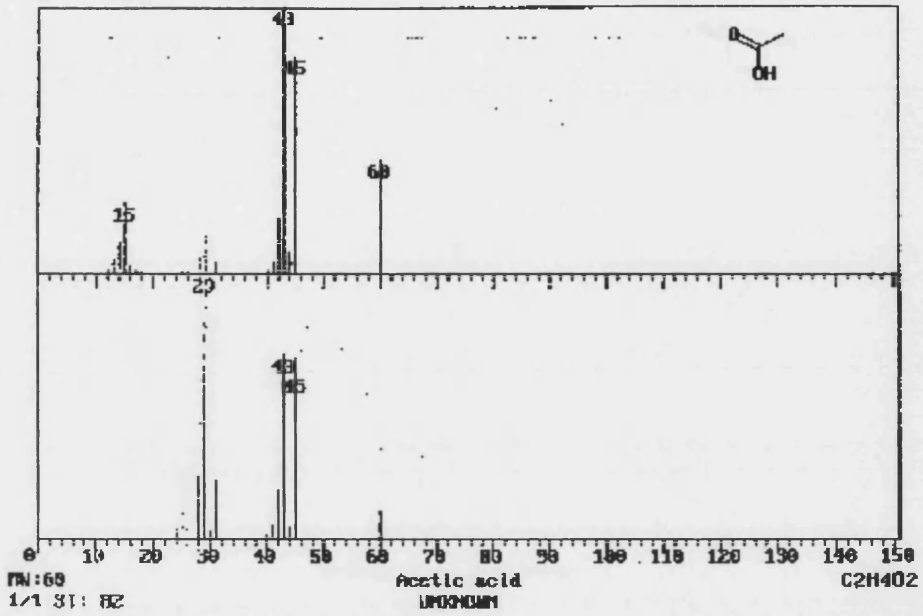
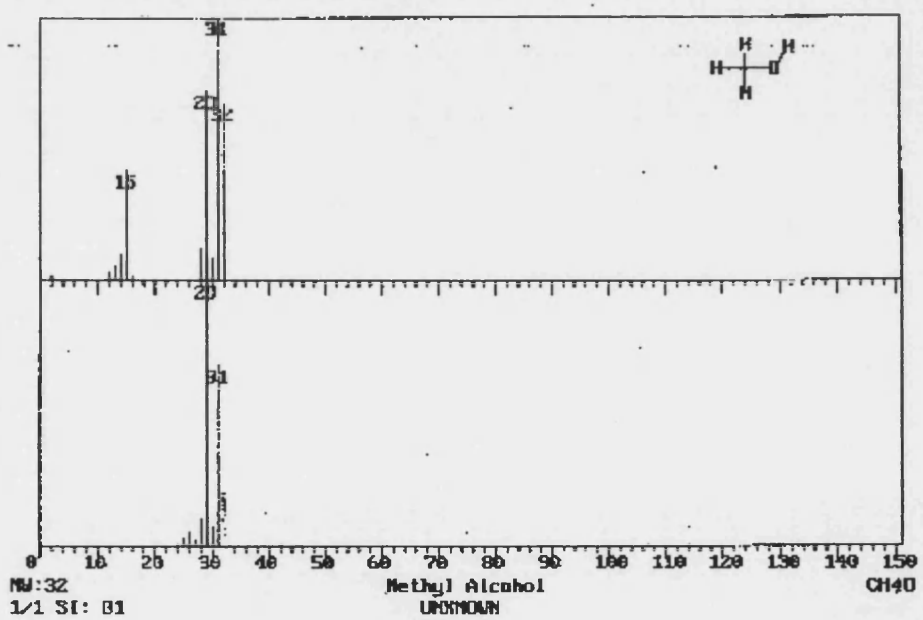
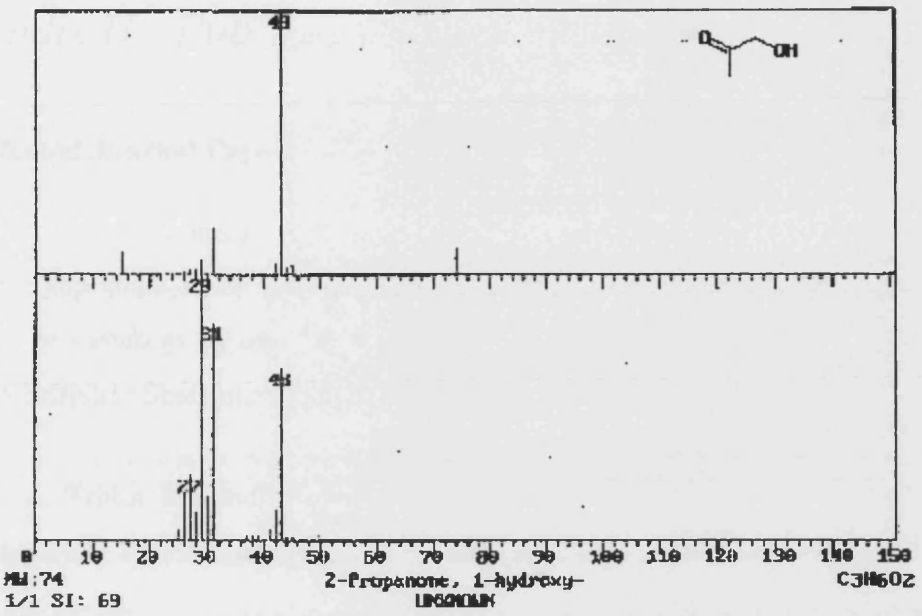
1/1 SI: 73
2-Furanmethanol
UNKNOWN
C5H6O2


MW:46
1/1 SI: 70
Formic acid
UNKNOWN
CH2O2


43
15
60
15
22
43
15
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
MW:60
Acetic acid
C2H4O2
1/1 SI: 82
UNKNOWN


31
29
32
15
29
31
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
MW:32
Methyl Alcohol
CH4O
1/1 SI: 81
UNKNOWN


43
O
OH
29
31
45
27
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
MW:74
1/1 SI: 69
2-Propanone, 1-hydroxy-
C3H6O2

Appendix H - Publications

H.I Refereed Journal Papers and Conference Proceedings

Trotin M., Scott J.A and Field R.W. (1997). Gaseous CO_2 stripping and recovery of volatile compounds from active beer fermentations. IN: *Engineering and food at ICEF 7: proceedings of the 7th International Congress on Engineering and Food,* Part 1, Sheffield : Sheffield Academic Press, B51-54.

Scott J.A., Trotin M. and Daugulis A.J. (1997). Removal of volatiles from high original gravity beer fermentations by gas (CO_2) stripping, *J. Am. Soc. Brew. Chem.*, **55** (1), pp. 16-19.

H.II Other Presentations

Trotin M., Scott J.A, Daugulis A.J., Field R.W and Shepherd S.H. (1996). Extraction and recovery of useful volatile organic compounds from beverage fermentation CO_2. *46th Canadian Chemical Engineering Conference*, Sept.29-Oct.2, Kingston, Ontario, Canada (oral presentation).

Scott J.A, Trotin M., Daugulis A.J. and Cooke D.E. (1995). Recovery for reuse of volatiles in CO_2 from beer fermentations. *Am. Soc. Brew. Chemist 61st Annual Meeting*, April 8-12, San Diego, California, US (poster presentation)

Removal of Volatiles from Very-High-Gravity Beer Fermentations by Gas (CO_2) Stripping

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ABSTRACT

J. Am. Soc. Brew. Chem. 55(1):16–19, 1997

The use of periodic forced gas (CO_2) stripping of ethanol and fusel oils from active very-high-gravity (1.080) beer fermentations has been shown to be a possible technique for both keeping ethanol levels low and manipulating the overall volatile balance of the beverage. Volatiles carried over in the gas stream were recovered by condensation (–8°C) and their extraction was shown to be related to medium concentration, gas flow rate, and fermentation temperature. The rate at which ethanol, isobutanol, isoamyl alcohol, and propanol were removed reflected their condensate-stripped wort concentration ratio and was linked to their respective volatiles. A rise in fermentation temperature from 17 to 22°C increased the volume stripping rate by over 100% and the relative quantity of ethanol removed by 30%. At the same fermentation temperature, raising the gas flow rate four times resulted in a threefold rise in the total volume extracted, but the ethanol concentration ratios remained similar.

Keywords: Beer, Carbon dioxide, Condensation, Stripping, Very high gravity, Volatile extraction

RESUMEN

El uso periódico de un desplazamiento forzado con gas (CO_2) del etanol y otros aceites de fusel de fermentaciones activas de mostos de alta gravedad (1.080) ha mostrado ser una posible técnica para mantener niveles bajos de etanol y para manipular el balance global de volátiles del producto. Los volátiles contenidos en el flujo de gas, fueron recuperados por condensación (–8°C) y se encontró que la extracción está relacionada a la concentración del medio, al flujo de gas y a la temperatura de fermentación. La velocidad a la cual el etanol, isobutanol, alcohol isoamilico y propanol fueron desplazados fue un reflejo de su relación de concentraciones condensado/cerveza y está ligado a su respectiva volatilidad. Unicremento en la temperatura de fermentación de 17 a 22°C incrementó la velocidad de desplazamiento en más de un 100% y la cantidad relativa de etanol desplazado en un 30%. A la misma temperatura de fermentación, pero aumentando la velocidad del flujo de gas 4.5 veces, se obtuvo un incremento de 3 veces en el volumen total desplazado, pero las relaciones de concentración de etanol permanecieron igual.

As one means of meeting the demand to provide tighter regulation of beverage production and also introduce modified organoleptic profiles, manipulation of the active fermentation is a potential option. One possible technique is the use of forced gas (CO_2) stripping to modify the balance of key components through continuous or periodic removal of ethanol and other volatile organic compounds (VOCs) (5,7). The process relies on mass transfer of volatile components from the liquid medium into the gas phase by a driving force proportional to the concentration gradient. Gaseous CO_2 may not necessarily be the most efficient extractor of beverage volatiles, but it is generated on-site as a copious, natural by-product of fermentation and hence represents a potentially vast reservoir of extractant.

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Publication no. J-1997-0102-04R.

Gas stripping of low-concentration volatiles from aqueous solutions, such as in air stripping of drinking water supplies, is usually modeled under steady state conditions by the two-film theory (4). Volatile mass transfer rate across the liquid-gas interface is related to the volatile's liquid concentration gradient, an approach that can be readily adapted to describe CO_2 stripping of volatiles from a fermenting wort:

$$J_v = K_L a(C_{v*} - C_v) \quad (1)$$

where J_v = volatile mass transfer rate (kg/m^{3}s); C_{v*} = volatile equilibrium concentration in the wort (kg/m^3); C_v = actual volatile concentration in the beer (kg/m^3); K_L = overall liquid mass transfer coefficient (m/s), and a = CO_2/liquid interfacial area per unit volume of wort (m^2/m^3). The value for overall liquid mass transfer coefficient, K_L, incorporates the diffusion resistance to mass transfer in both phases and is related to the volatile's local gas and liquid mass transfer coefficients, k_G and k_L, respectively, and its Henry's law coefficient, H_v, by:

$$1/K_L = (1/k_L) + (1/k_G H_v) \quad (2)$$

and the overall CO_2 phase mass transfer coefficient, K_G, can be similarly defined as:

$$1/K_G = (1/k_G) + (H_v/k_L) \quad (3)$$

When a constituent has a relatively large Henry's constant, such as the volatile components in the wort, then K_L and K_G will be dominated by resistance to transfer through the liquid (k_L), such that:

$$K_L \approx k_L \text{ and } K_G \approx k_L/H_v \quad (4)$$

Most current commercial applications for volatile removal from beverages are for post-fermentation production of ethanol-depleted products (9) and require "add-off" separate stage equipment (7). In contrast, the use of forced-gas stripping provides the option of continuous and/or periodic extraction, which can manipulate or modify the volatile balance of the fermentation as it progresses. Furthermore, through continuous removal, ethanol concentrations can be kept below yeast inhibitory levels such that very-high-gravity media can be fully fermented without premature cessation.

By reducing the temperature of the gas stream after it leaves the fermenter, extracted components can be condensed-out in a reasonably concentrated form. Recovery of key volatiles from the condensate, such as by use of recyclable extractants (6), would allow them to be fractionated and/or returned to the stripped medium. For example, these "natural origin" VOCs, if collected, could be added back to enhance immediacy and strength of key perceived flavor profiles. Alternatively, components could be used to modify/develop organoleptic trends (e.g., addition of selected alcohols collected from one fermentation into another to promote exaggerated production of desirable esters).

In this article, principal parameters that influence both volatile extraction rate and subsequent condensate concentration through periodic stripping with CO_2 are examined. Using a very-high-gravity (1.080) beer style medium, the factors assessed include medium temperature and volatile concentration as well as gas flow rate.

EXPERIMENTAL

Fermentation

Into sterile 500-ml shaker flasks were added 100-ml aliquots of YPD medium (Difco Laboratories), which were each inoculated with a loop-full of a *Saccharomyces cerevisiae* ale strain (NCYC 1236, National Collection of Yeast Cultures, Norwich, England). The flasks were then placed in a shaking incubator for 24 hr at 30°C, after which was added 400 ml of a fortified wort medium that consisted of malt (70 g/L), hops (5.25 g/L), barley grains (11.25 g/L), and brewing liquid sugar (180 g/L, Edme Ltd., Manningtree, England) in distilled water (OG 1.080 ± 0.001, pH 5.0 ± 0.1).

After a further 24 hr in the shaking incubator, the media obtained contained between 1.8 and 2.8 × 10^9 cells/ml (>98% viable). Sufficient aliquots of the prepared inoculum were then transferred into temperature-controlled 22-cm-diameter 10-L glass culture vessels (Fisher Scientific, Loughborough, England) holding 8.6 L of fresh fortified wort to give an initial 2.7 × 10^7 cells/ml. Total cell counts and percent viability were assessed using a Neubauer hemacytometer with diluted samples (10:1 and 100:1) in glucose/Ringers buffer and an equal volume of methylene blue (dead cells were taken as those that took up the stain).

For each experimental run, equal numbers of fermenters were set up with half acting as controls (no gas circulation) and the others operated with regulated (0.0–0.5 L of CO_2 per liter of medium per minute) addition of CO_2 from a cylinder (99.8% pure) through a 9-cm-diameter ring-sparger located in the fermenter bottom. Each fermenter was fitted with gas tubing from the top to a condenser (operated at –8°C) for removal of volatiles carried over in the gas stream. Fermentations were carried out at either 17 or 22 ± 0.5°C. For the initial stripping period (starting on day 4), 0.5 ml of food-grade antifoam was added (Dow Corning 1520, BDK Poole, England) and before each subsequent stripping period, 0.25 ml were added. The results presented are averages of three complete runs at each temperature; for calculating rate of ethanol extracted per unit volume of medium they are adjusted for volume losses from the fermenters.

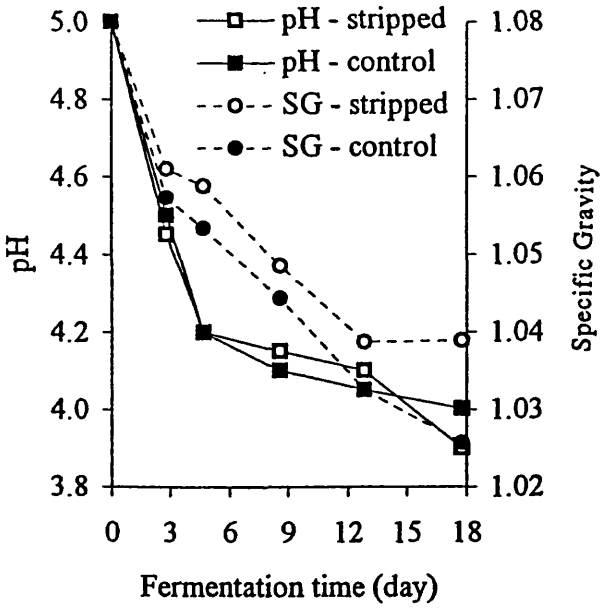

Fig. 1. Media pH and specific gravity (SG) for control and stripped beer fermentations at 22°C (stripping at 0.5 L of CO_2 per liter of medium per minute on days 4–7, 9–10, and 14–17).

Analysis of Ethanol in Beer Media and Ethanol and Fusel Oils in the Condensate

A Hewlett Packard 5890 series II gas chromatograph equipped with an HP 7673 automatic sampler, a flame ionization detector, and a HP 3396 series II integrator was used. The column was a 50 m × 0.22 i.d. BP20 capillary column (SGE Ltd., Milton Keynes, England) with a film thickness of 0.25 µm. Triplicate samples were diluted to 1:100 before ethanol analysis. The injection size was 1 µl and the oven temperature was isothermal at 115°C for 5 min. For volatiles in the condensate, 1-butanol was used as an internal standard and the oven temperature was programmed at 40°C for 6 min, raised to 100°C at 5°C/min, and then held for 1 min. For all the analyses, injector and detector were maintained at 200 and 250°C, respectively, and helium was used as the carrier gas at a flow rate of 1. 5 ml/min.

Analysis of Fusel Oils in Beer Media

A Hewlett Packard 5790 A Series gas chromatograph equipped with a flame ionization detector and interfaced with a LDC/Milton Roy CI-10B integrator was used with a 2.5 m long, 3 mm i.d. stainless steel column packed with Chromosorb 101 (mesh size 80–100) (Phase Separations Ltd., Queensferry, Wales). After sample injection (1 µl), the column temperature was held at 150°C for 20 min. The injector and detector were maintained at 200 and 250°C, respectively. The helium carrier gas flow was 40 ml/min, and 1-butanol was used as an internal standard.

RESULTS AND DISCUSSION

Forced gas stripping has been used with alcoholic cider (7), and the fermentation volume was found large enough (35–40 L) to generate sufficient CO_2 so as to be "self-sufficient." That is, volatiles were condensed out and the gas recirculated (pumped) back to the bottom of the fermenter without need for a supplementary supply. With the 8.6-L beer fermentations used in this work, it was considered that the quantities of gas produced were not sufficient for sensible recirculation using the equipment available. As a consequence, the CO_2, which was supplied to ring-spargers located at the bottom of the fermenters, was from gas cylinders and was not recirculated after passing through the condensers.

With the cider fermentations, a greater reduction in the medium pH was recorded in stripped media than in the controls, due presumably to a rise in background CO_2 levels from around 0.10% w/w (control) to 0.37% w/w (stripped) (8). With stripped ciders, the pH fell to a minimum of 2.8 compared with 3.15 in non-stripped controls, although for both types of fermentation a gradual rise in pH started at around the halfway stage as the organic acid level was reduced through esterification. Elevated levels of CO_2 have been reported to induce changes in yeast physiology (e.g., an increased cell size [8]) and metabolism (e.g., reduced fusel oil production [3]). In the case of the gas-stripped very-high-gravity (1.080) beer fermentations, CO_2 concentrations were not directly measured. However, the pH profiles remained similar to the non-stripped controls throughout fermentation (Fig. 1), indicating that under the conditions used, the increased exposure to CO_2 did not adversely affect the pH balance.

In terms of the yeast populations after a relatively long fermentation period of 17 days, the average numbers in the stripped and control fermentations were 1.7 ± 0.2 × 10^8 and 1.3 ± 0.2 × 10^8

cells/ml, respectively. However, if the stripped fermentations were adjusted for volume loss (i.e., the quantity of collected condensate), then the average values were similar to the control at 1.4 ± 0.2 × 10^8 cells/ml. As there was also little difference after 17 days between cell viability in stripped and control fermentations (estimated at between 70 and 75% of the total cell numbers), variations in VOC levels between the control and stripped fermentation media are more likely initiated by physical extraction rather than by modified yeast activity.

Changes in media ethanol concentrations along with the levels in the collected condensate are shown in Figure 2. Fermentations were allowed to proceed for extended periods and with three separate stripping periods (days 4–7, 9–10, and 14–17). In the control, by day 17 ethanol concentration had reached 6.8% v/v, but due to the periodic forced CO_2 stripping, was reduced to 3.4% (v/v) in the stripped media. This significantly lower ethanol level toward the end of fermentation is reflected by a higher specific gravity (Fig. 1).

The higher the ethanol concentration in the beer, the greater the available driving force for transfer between phases, which should result in a higher condensate concentration. This is confirmed in Figure 3 by the direct relationship between the two concentrations at two different fermentation temperatures. In addition, the rate of ethanol production within the fermentation is also key to dictating overall quantities collected in the condensate. For example, at 22°C, medium ethanol concentrations were similar in the middle of two stripping periods, days 10 and 16, at around 3.95% (v/v), but the production rates, estimated from the control fermentation, were notably different at 3 and 1.6 cm^3 per liter of medium per day, respectively. This is reflected in the stripped fermentations by a period of relatively stable ethanol concentration between days 9 and 10 (Fig. 2), but marked falls in both condensate and medium levels between days 14 and 17 as a consequence of the fall in average production.

There are other process factors that can also regulate volatile removal rate, such as medium temperature. In terms of temperature, at the same CO_2 stripping rate (0.5 L of CO_2 per liter of medium per minute) with 17°C fermentations, the condensate recovery rate was 56% less than that at 22°C (i.e., a fall from 16 to 7 cm^3 per liter of medium per day). At 17°C the level of ethanol in the condensate was also reduced.

For example, at medium levels of around 3% (v/v), the condensate ethanol levels at 17 and 22°C were 7.0 and 9.5% (v/v), respectively (Fig. 3). Although at this stage the data is limited to two temperatures, the results obtained were consistent. That is, as would be expected, a rise in temperature had a relatively greater impact on the more volatile components in the beverage by increasing their extraction rate.

Another process factor to consider is gas flow rate. The number of bubbles per unit volume of medium is increased as gas flow rate is raised with a commensurate rise in both the total interfacial area and gas hold-up in the beer. From equation 1, the consequence should be an increase in the overall volume of volatiles removed per unit time, although the condensate-stripped wort volatile concentration ratios should be similar. For example, in fermentations operated at 17°C, increasing the flow rate from 0.1 to 0.5 L of CO_2 per liter of medium per minute resulted in a threefold increase in the rate of condensate removed (from 2.3 to 7 cm^3 per liter of medium per day). It should be noted, however, that in terms of a large-scale unit, any increase in flow rate has to be considered against the increased risk of foaming and higher pumping costs, both of which are likely to impose restrictions on the maximum practical rate.

The changes in condensate levels of the three higher alcohols at 22°C appear to follow those of ethanol (Fig. 4), which suggests that fusel oil and ethanol production rates were linked throughout the fermentation stripping process. After 14 days (6.2%, v/v, ethanol), the three measured fusel oils in the control fermentations were at an average of 17 mg/L for propanol, 50 mg/L for isobutanol (2-methyl, 1-propanol), and 223 mg/L for isoamyl alcohol (3-methyl, 1-butanol). While the propanol level is similar, the isobutanol and isoamyl levels are around 24 times greater than those quoted for beers with ethanol levels between 3 and 4%, v/v (2). Whereas at the same time, the average levels for propanol, isobutanol, and isoamyl alcohol in the stripped medium (4.6%, v/v, ethanol) were similar to 3–4% (v/v) beers at 10, 30, and 118 mg/L, respectively, and for the collected condensate 20, 86, and 434 mg/L.

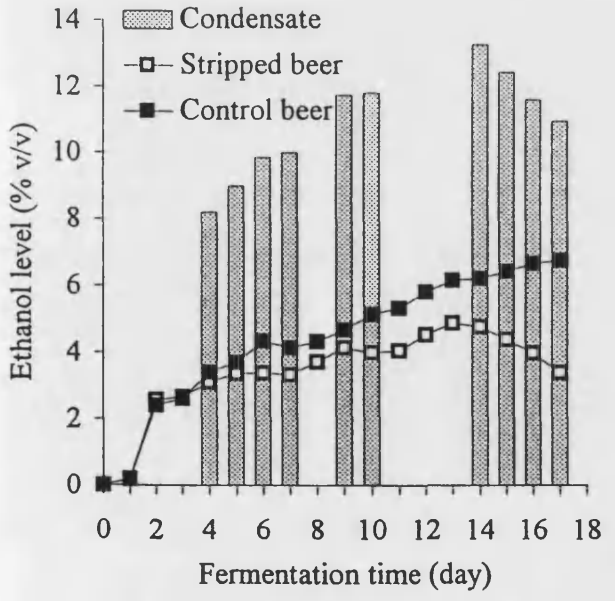

Fig. 2. Ethanol levels in control and stripped beer fermentations at 22°C and collected stripped condensate (stripping at 0.5 L of CO_2 per liter of medium per minute on days 4–7, 9–10, and 14–17).

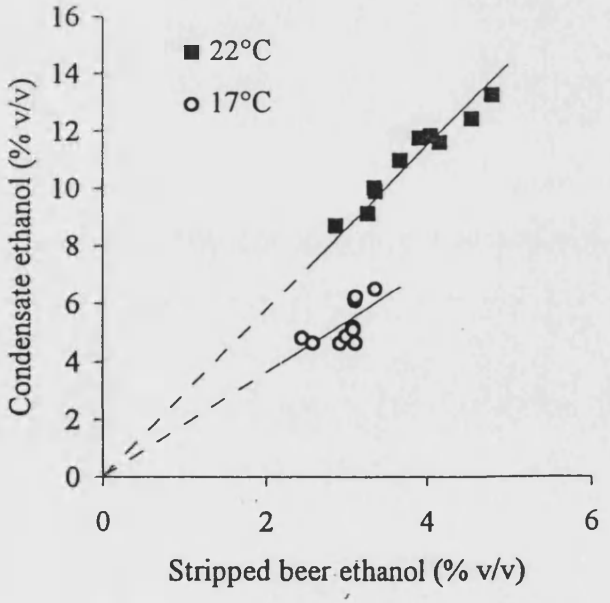

Fig. 3. Relationship between stripped beer medium and condensate ethanol levels at fermentation temperatures of 17 and 22°C (stripping at 0.5 L of CO_2 per liter of medium per minute on days 4–7, 9–10, and 14–17).

TABLE I
Ratio of Volatile Concentrations in the Condensate and Stripped Wort

Fermentation Day	Ethanol	Propanol	Isobutanol	Isoamyl Alcohol
6	2.88	2.18	3.58	4.77
10	2.61	2.20	3.18	5.06
14	2.71	2.13	3.31	4.82
17	2.95	1.98	3.51	5.52

The greater reduction over time in isobutanol and isoamyl alcohol when compared with both ethanol and propanol is a result of higher overall mass transfer coefficients (K_L). Although only a limited amount of data on fusel oil content within stripped beer media was obtained, the higher K_L values can be also illustrated by the calculated condensate-stripped wort medium volatile concentration ratios (Table I). The values for isobutanol and isoamyl alcohol were consistently greater than for ethanol or propanol, that is stripping removed a relatively higher proportion of the former two compounds.

This is reflected in dimensionless Henry's law constants (H_v):

$$H_v = y_i/x_i \quad (5)$$

where y_i and x_i are mole fractions of the volatile in the gas and liquid phases. The higher the value of H_v, the more volatile and hence more easily stripped the wort component should be. Estimates of H_v were subsequently obtained from published vapor-liquid equilibrium data (1) and were 10.1 ± 0.5, 10.8 ± 0.3, and 23.7 ± 2 for ethanol, propanol, and isobutanol (data not available for isoamyl alcohol), respectively. Caution needs to be exercised as to the actual magnitude of these values as they were determined from data on binary mixtures whereas with a CO_2-wort system this is clearly not the case. The similar H_v and concentration ratio data (Table I) for ethanol and propanol, however, suggested statistically little difference in behavior in a stripped system, whereas the notably higher H_v value for isobutanol is reflected in a greater concentration ratio, indicating a more readily stripped component.

These type of data could, therefore, provide useful information on how beer fermentations can be manipulated in terms of determining the expected relative removal of volatiles and could be exploited in the future in extraction of unwanted as well as desirable beverage components. For example, by initiating short-term extraction during periods of high concentration of certain volatiles (perhaps unwanted sulfur compounds), an exaggerated removal of them could be achieved.

CONCLUSIONS

Removal of ethanol and other volatiles by forced CO_2 circulation followed by recovery from the gas stream by condensation is relatively straightforward. It also offers several potential process advantages over existing extraction methods, including use of an on-site "natural," abundant, and essentially free extractant, no application of heat to the beverage, and the option of continuous or periodic extraction.

Through controlled gas stripping at key times during beer fermentation, it is possible to manipulate the level of volatiles within the beverage medium, although the rate of extraction will be volatile dependent. Another intriguing option is the potential of removing unwanted compounds such as sulfur-containing volatiles, a possibility currently under consideration. That is, if periods of maximum production are identified, then a short burst of gas stripping could be targeted to reduce potential off-flavors.

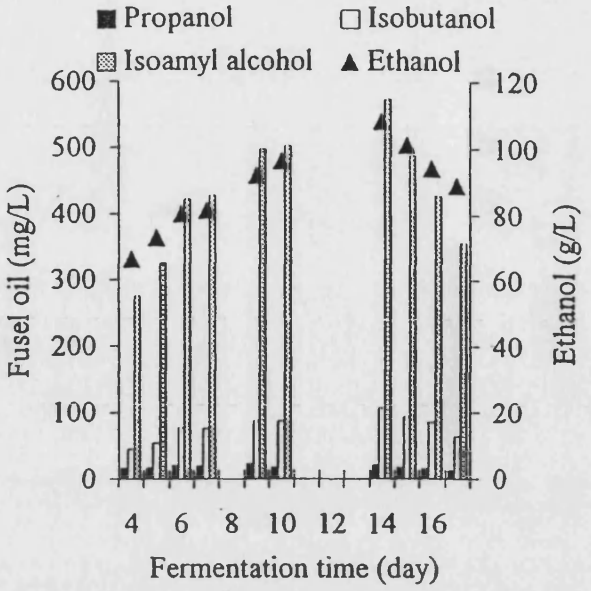

Fig. 4. Stripped beer condensate volatile levels (22°C fermentation stripped at 0.5 L of CO_2 per liter of medium per minute on days 4–7, 9–10, and 14–17).

ACKNOWLEDGMENTS

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